STUDIES IN HÆMOLYSIS

BY

RAI UPENDRANATH BRAHMACHARI, BAHADUR,

M.A., M.D., PH.D.,

TEACHER OF MEDICINE AT THE CAMPBELL MEDICAL SCHOOL, CALCUTTA;
FELLOW OF THE ROYAL SOCIETY OF MEDICINE; FELLOW
OF THE CALCUTTA UNIVERSITY; EXAMINER IN
MEDICINE AND PHYSIOLOGY TO THE
CALCUTTA UNIVERSITY

UNIVERSITY OF CALCUTTA,
No. 339
REGISTRAR'S OFFICE.

SECOND EDITION

CALCUTTA
PUBLISHED BY THE UNIVERSITY OF CALCUTTA
1913

CENTRAL LIBRARY

BCU 1844

Printed by Atulchandra Bhattacharyya,

AT THE

CALCUTTA UNIVERSITY PRESS

Senate House, Calcutta.

GS 2372

TO

THE SACRED MEMORY

OF

MY FATHER

Dr. NILMANI BRAHMACHARI

WHOSE DEVOTION TO DUTY AND NOBILITY OF CHARACTRR

ENDEARED HIM TO ALL WHO KNEW HIM

AND

WHOM I NEVER KNEW TO DO AN UNJUST ACT

OR CHERISH AN UNJUST THOUGHT

AND

WHO STILL LIVES AS THE

INSPIRING GENIUS TO GUIDE ME IN MY LIFE

I DEDICATE THIS WORK AS THE

HUMBLE TRIBUTE OF A LOVING AND GRATEFUL SON

PREFACE TO THE SECOND EDITION.

The study of the physical aspects of hæmolysis has been carried on by me for the last few years. So far as I am aware, very little work has been done on the lines indicated in this treatise. Since the first edition was published in 1909, I have continued the study of the subject and as a result of these further labours, some new facts have been discovered which are incorporated in the present edition. Besides the older portions of the text have been thoroughly revised, re-written and condensed. Among the new facts brought to light may be mentioned :—the fallacy of the hæmosozoic value of blood, as worked out by Sir A. E. Wright, for the determination of complete hæmolysis ; the hæmoglobin-value of the resistant erythrocytes ; the freezing points of the unhæmolysed corpuscles ; the law regulating hæmolysis, and the properties of salted erythrocytes. These latter were very interesting and led me to doubt whether the view generally held that the erythrocytes are impermeable to NaCl holds good in the case of blood treated with saturated or half-saturated NaCl solution, and on chemical analysis it was found that in every case some amount of NaCl was absorbed by the erythrocytes by a process which is probably allied to *adsorption*. The properties of the salted erythrocytes have been described in detail, as, so far as I am aware, they have not been noted by any previous observer.

By the study of the hæmoglobin-value of the resistant erythrocytes, I have discovered a new method of testing blood which may be of use in hæmatology.

Among other subjects studied by me are the resistance of erythrocytes to hæmolysis in some forms of anæmia, hæm-alkalinity and hæmo-salinity, effects of evaporation on the resistance of erythrocytes to hæmolysis, mechanism of crenation of erythrocytes, and spontaneous hæmolysis.

I have expressed my views in detail about the mechanism of hæmolysis by saline solutions and the constitution of erythrocytes as revealed from this phenomenon. I shall wait, with much interest, for the criticism of my views, from physiologists who may be working in this line.

My grateful thanks are due to Major McCay, I.M.S., Professor of Physiology, Medical College, Calcutta, at whose suggestion I first took up the study of the subject of hæmolysis for the Ph. D. degree of the Calcutta University. I am also under deep obligation to Professor Benjamin Moore, F. R. S., of the Liverpool University for his kindly publishing some of my results in the Bio-Chemical Journal and for his kind encouragement in my researches.

CALCUTTA;
January, 1913.

U. N. BRAHMACHARI.

CONTENTS.

CHAPTER I.

Complete hæmolysis—a critical study of Sir A. E. Wright's method of determining complete hæmolysis.

Sir A. E. Wright and Kilner[1] in describing a new method of testing blood and the urine point out that it depends upon the determination, in each case, how far a standard suspension of the erythrocytes requires to be diluted before complete hæmolysis is obtained. They state that "for this purpose we make a series of progressive dilutions of a deci-normal sodium chloride solution.....We now take up into our capillary tube one volume of each of these dilutions mixing in each case with one volume of the suspension of the corpuscles. *Holding at an arm's length the capillary pipette which contains the series of mixtures and disposing the tube so that the light may fall upon it obliquely, we take cognisance of the hæmolytic effect. Opacity and a bright appearance referable to the reflection of light to the eye indicate incomplete transparency and a dark colouration due to the absence of reflection indicate complete hæmolysis.*"

Later on, Wright and Ross[2] modified the above method by pointing out that instead of going through the need of making

---

[1] Wright and Kilner,
Lancet, April 2nd, 1904.

[2] Wright and Ross,
Ibid, October 21st, 1905.

a preparation of a suitable suspension of the red corpuscles, all that is required is to take a single measured volume of the blood and to mix with it in each case two volumes of progressive dilutions of deci-normal sodium chloride solution.

Working in the above way, Wright and Ross conclude that the average European blood hæmolyses *completely* with two parts of $\frac{N}{5\cdot5}$ NaCl solution.

From the above, it will be seen that it is assumed that it is possible to bring about complete hæmolysis by mixing one volume of blood with two volumes of a sufficiently dilute solution of sodium chloride and that the point at which complete hæmolysis is reached can be determined by letting light fall obliquely upon capillary tubes containing the blood, complete hæmolysis being supposed to be arrived at, when there is dark colouration of the blood and there is no bright appearance to be seen in it.

My observations on the blood of healthy medical students lead to different conclusions from those of Sir A. E. Wright. By treating normal blood with two volumes of $\frac{N}{100}$ NaCl solution as well as with two vols. of distilled water, which may be represented by $\frac{N}{\infty}$, I have succeeded in demonstrating that in none of these is complete hæmolysis obtained.

I consider that the most accurate conception of complete hæmolysis should be that the blood supposed to be completely hæmolysed should be perfectly transparent and leave no sediment or if it is not perfectly transparent, it should give, on centrifugalisation, a sediment which when thoroughly washed with an *inactive* fluid should not be red. Further, it should not shew the presence of hæmoglobin-containing erythrocytes,

which can be stained with proper stains. By an *inactive* fluid is meant a fluid which will dissolve any free hæmoglobin but has no action on the erythrocytes and cannot, therefore, dissolve the hæmoglobin contained in them.

To determine whether the sediment is red or not, the supernatant fluid should be pipetted off and treated with a solution of NaCl which cannot cause any more hæmolysis in the blood under consideration. The mixture is then centrifugalised again and the sediment separated and treated in the same way as before and if after a sufficient number of washings, it is found that the supernatant fluid at the top is colourless after centrifugalisation and the sediment is red and not colourless, then it is evident that complete hæmolysis has not taken place; the sediment may be further tested for the presence of hæmoglobin-containing corpuscles and then stained with a proper stain to shew the presence of stained erythrocytes. If, on the other hand, the sediment is colourless, then complete hæmolysis has taken place. Under ordinary circumstances, an $\frac{N}{10}$ NaCl solution will serve the purpose of the inactive fluid mentioned above, as it does not hæmolyse either human or the rabbit's or the frog's blood. We shall, however, see, later on, that $\frac{N}{10}$ NaCl solution cannot always be used for the above purpose, as, for instance, when the blood has been previously treated with a saturated NaCl solution.

We began our investigations by testing different specimens of blood from the students of the Campbell Medical School, Calcutta. The blood of a large number of students was examined in the above way, the diluting fluid being either distilled water or $\frac{N}{100}$ NaCl solution, generally the latter. In none of

these cases did I observe complete hæmolysis conforming to the definition given above. In other words, I always obtained a red sediment after the blood was treated in the way described above. At the same time the dark colouration described by Wright was generally obtained by mixing the blood with two vols. of $\frac{N}{40}$ to $\frac{N}{50}$ NaCl solution.

That the red sediment described in the above experiments contain undissolved erythrocytes can be shewn in the following way :—

(1) The sediment though insoluble in $\frac{N}{10}$ NaCl is dissolved by being repeatedly washed with $\frac{N}{100}$ NaCl solution or distilled water.

(2) The sediment shews the presence of hæmoglobin-containing erythrocytes under the microscope.

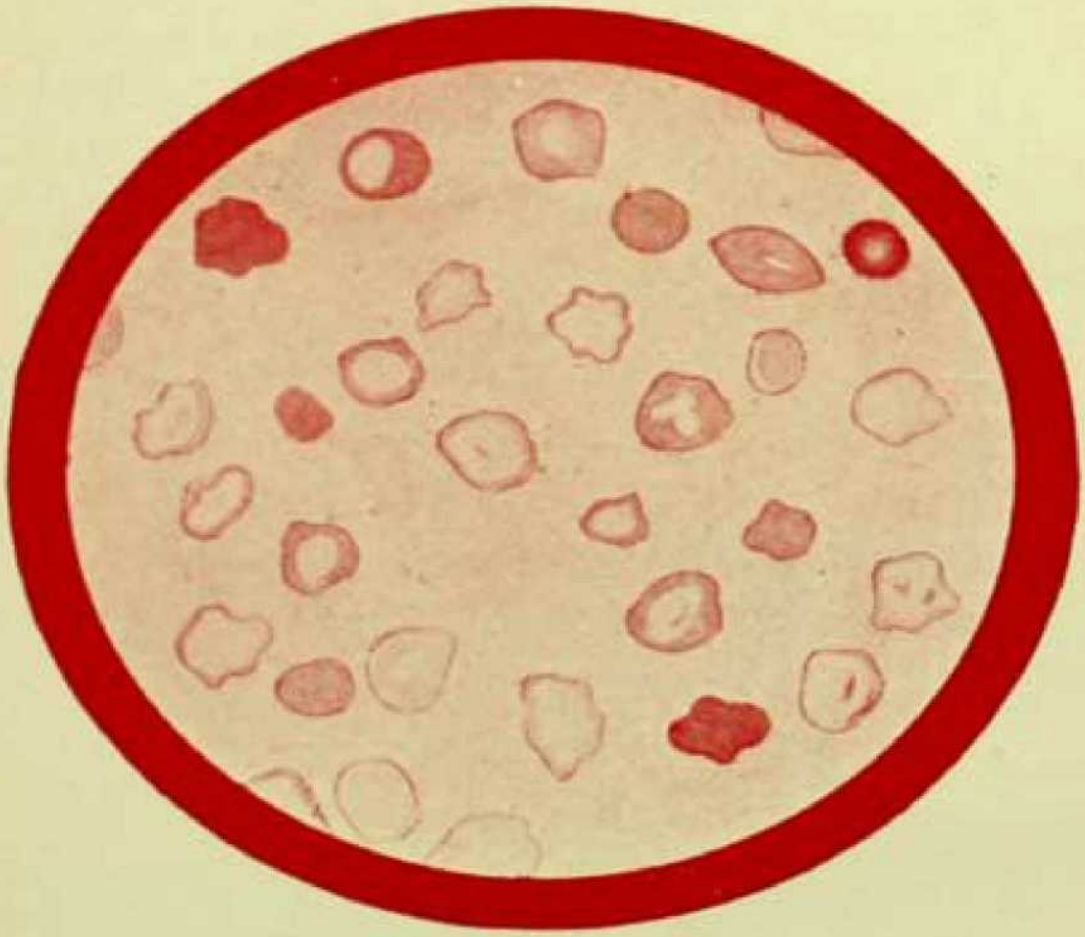
(3) The sediment when stained with a proper stain shews the presence of stained erythrocytes.

In some of my cases, I put the mixture of blood with distilled water as well as the mixture of blood with $\frac{N}{100}$ NaCl solution for nearly 24 hours in small corked tubes and it was found that complete hæmolysis had not taken place even after this period, the temperature of the room being 29° C. in the day.

The question now arises as to how many parts of distilled water or $\frac{N}{100}$ NaCl solution can completely hæmolyse one part of human blood. I have made dilutions of blood several times with one, two, up to nine parts of distilled water as well as $\frac{N}{100}$ NaCl solution and have found the red sediment in all of them even after repeated washing of the sediment with $\frac{N}{10}$ NaCl

CENTRAL LIBRARY

PLATE I.



Resistant corpuscles from a mixture of one part of blood and forty parts of distilled water.

(Page 5.)

solution. The sediment also shewed the presence of hæmoglobin-containing erythrocytes which easily took eosin stain. In a few cases I diluted a specimen of blood with 40 parts of distilled water and kept the mixture for 12 hours in a small tube and could detect the presence of hæmoglobin-containing erythrocytes which took eosin stain very well. (See plate No. I.)

The corpuscles that are found in the red sediment described above and which contain hæmoglobin, will, in future, be called, for the sake of brevity, *resistant corpuscles*.

The resistant corpuscles can be fixed in absolute alcohol or methyl alcohol and stained with eosin.

The diagram in plate No. II shews the resistant corpuscles obtained by mixing one part of blood with two parts of $\frac{N}{100}$ NaCl solution and then keeping the mixture undisturbed for one hour at the temperature of the room (29° C.). A similar phenomenon is seen when blood is treated with 9 vols. of $\frac{N}{100}$ NaCl except that the resistant corpuscles are much fewer and the destructive changes noticed in them are more marked.

We thus see that complete hæmolysis cannot be brought about by mixing one volume of blood with even nine vols. of distilled water or $\frac{N}{100}$ NaCl solution. The dark colouration described by Wright and others is, however, generally obtained when the blood is mixed with two parts of $\frac{N}{40}$ to $\frac{N}{50}$ NaCl solution.

The point at which the dark colouration is obtained may, for the sake of convenience, be called *Wright's hæmolytic point*. It does not, however, represent the point of absolutely complete hæmolysis. This hæmolytic point differs, in the case of the Bengalees, from that of Europeans as determined by Wright and

others. In the case of the latter, Wright found it to be reached at $\frac{N}{35}$ NaCl, while in the case of the Bengalees it was reached in my observations at $\frac{N}{40}$ to $\frac{N}{50}$ NaCl. This closely agrees with the observations of Major McCay[1].

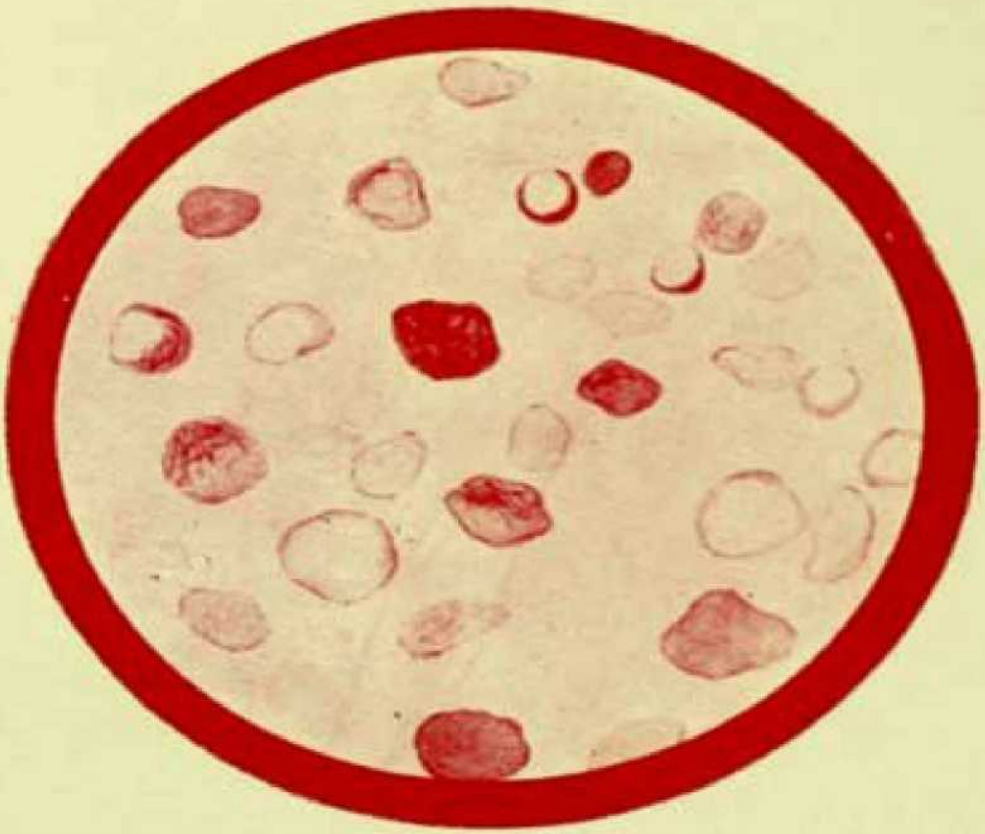
---

[1] McCay,

Lancet, June 1st, 1907.

CENTRAL LIBRARY

PLALE II.



Resistant corpuscles from a mixture of one part of blood and two parts of $\frac{N}{100}$ NaCl solution.

(Page 5.)

CHAPTER II.

Behaviour of the erythrocytes towards hyposmotic solutions of NaCl—the curve of hæmolysis with hyposmotic NaCl solutions.

In the former chapter, I have pointed out that complete hæmolysis cannot be brought about by mixing one volume of blood with two vols. of a progressive dilution of NaCl solution, however high the dilution may be. I have observed that the degree of hæmolysis is very sudden from $\frac{N}{20}$ to $\frac{N}{30}$ NaCl solutions, and from $\frac{N}{30}$ to $\frac{N}{45}$ or $\frac{N}{50}$ it is somewhat gradual. From $\frac{N}{50}$ upwards, the amount of hæmolysis increases still more slightly with the higher dilutions. With $\frac{N}{15}$, generally, there is no hæmolysis, while with $\frac{N}{20}$ it is faint or sometimes absent. This latter fact does not quite agree with the observations of Sir A. E. Wright. The table on page 8, shews the degree of hæmolysis obtained by mixing one volume of blood with 2 vols. of $\frac{N}{10}$ to $\frac{N}{30}$ NaCl solutions.

	Blood + 2 vols. of $\frac{N}{10}$ NaCl.	Blood + 2 vols. of $\frac{N}{15}$ NaCl.	Blood + 2 vols. of $\frac{N}{20}$ NaCl.	Blood + 9 vols. of $\frac{N}{30}$ NaCl.
1			Slight H	Marked H
2			Do.	Do.
3			Do.	Distinct
4			Do.	Do.
5			Very slight	Do.
6			Slight	Marked
7			Do.	Do.
8			Do.	Do.
9			Faint	Distinct
10			Slight	Marked
11			Very faint	Distinct
12			Do.	Marked
13			Do.	Do.
14			Do.	Do.
15			Faint	Do.
16	Nil H	Nil H	Very faint	Do.
17	Do.	Do.	Do.	Distinct
18	Do.	Do.	Do.	Do.
19	Do.	Do.	Nil	Do.
20	Do.	Do.	Do.	Marked
21	Do.	Do.	Do.	Do.
22	Do.	Do.	Very faint	Do.

H = Hæmolysis.

On page 8 in the heading of the 5th Column *read* Blood + 2 vols. *for* Blood + 9 vols.

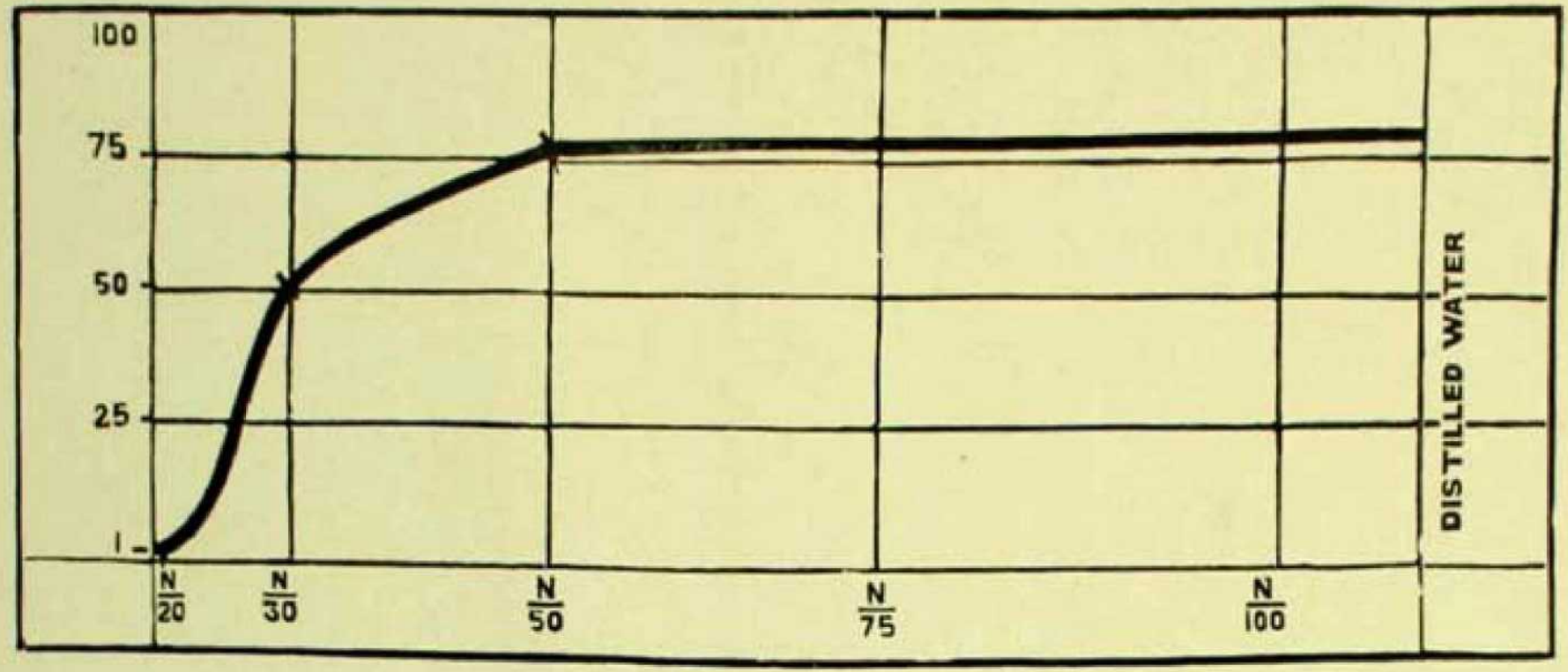


Fig. 1

The curve of hæmolysis with hyposmotic NaCl solution, the curve being drawn by estimating the amount of hæmoglobin in 20 cb. mm. of the supernatant fluid from the centrifuged mixture.

(Page 19)

The method by which I determined *quantitatively* the amount of dissolved hæmoglobin in a mixture of blood and NaCl solution was as follows :—

A definite volume of the clear supernatant fluid, generally 20 cb. mm., was taken from the above mixture and the amount of hæmoglobin in it was estimated by means of a Haldane's Hæmoglobinometer. The following table illustrates the point :—

Amount of dissolved hæmoglobin in 20 cb. mm. of the supernatant fluid obtained after centrifugalisation of a mixture of blood and 2 vols. of $\frac{N}{X}$ *NaCl solution.*

(X = any whole number from 20 to 100.)

$\frac{N}{20}$	$\frac{N}{30}$	$\frac{N}{40}$	$\frac{N}{50}$	$\frac{N}{60}$	$\frac{N}{100}$	Distilled water.
1%	50%	68%	75%	77%	78 %	80 %
1%	55%	70%	75%	—	78 %	82 %

In the above way, I have made out a curve which I have designated the *curve of hæmolysis with hyposmotic NaCl solutions.* (See Fig. I.)

From this curve, it will be seen that the very beginning of hæmolysis starts with $\frac{N}{20}$ NaCl solution. Then, as already mentioned, the degree of hæmolysis increases suddenly from $\frac{N}{20}$ to $\frac{N}{30}$ NaCl solution. This sudden increase is generally supposed to be due to the following cause :—When blood is treated

with a physiologically normal NaCl solution, there is no hæmolysis nor any change in the volume of the corpuscles. When the blood is treated with a slightly more dilute NaCl solution the erythrocytes swell up but do not allow the escape of hæmoglobin to take place. With $\frac{N}{20}$ NaCl the first beginning of hæmolysis is seen. But with $\frac{N}{30}$ more water enters the erythrocytes, most of which now become very much over-distended, and their walls rupture and allow a large amount of hæmoglobin to escape. Hence the sudden increase of hæmolysis from $\frac{N}{20}$ to $\frac{N}{30}$ NaCl solution. The very slight hæmolysis that is shewn when blood is treated with $\frac{N}{20}$ NaCl is probably due to the rupture of the few corpuscles that are least capable to bear the tension of distension. I shall, however, shew, later on, that *osmosis and rupture* alone cannot explain the whole phenomenon of hæmolysis by hyposmotic NaCl solutions.

CHAPTER III

Relative Hæmoglobin-value of the resistant erythrocytes during hæmolysis.

The relation of the amount of hæmoglobin in the resistant corpuscles to the total amount in a sample of blood under examination appears to me, from observations in health and desease, to be a matter of considerable importance, and I would suggest that this be called the *relative hæmoglobin-value of the resistant erythrocytes.* It may be expressed as the quotient obtained by dividing the amount of hæmoglobin in the resistant corpuscles by that of the total blood.

The method by which the amount of hæmoglobin in the resistant corpuscles was estimated is described as follows: In all cases the blood is hæmolysed with two parts of N/50 saline solution or destilled water. After thoroughly mixing 5 cb. mm. of the blood with 10 cb. mm. of N/50 saline or distilled water the mixture is centrifugalised as thoroughly as possible and the sediment at the bottom is thoroughly washed with N/10 saline till the supernatant fluid at the top is perfectly colourless. The sediment is now dissolved in a small quantity of distilled water with the addition of a drop or two of chloroform and then the amount of hæmoglobin is estimated by Haldane's Hæmoglobinometer. In those cases in which the amount of hæmoglobin in the resistant corpuscles is less than 10 per cent., 10 or 20 cb. mm. of blood is taken and then treated with 20 or 40 cb. mm.

of the hæmolysing fluid and the amount of hæmoglobin in the resistant corpuscles is then estimated. This number divided by two or four, as the case may be, gives the amount of hæmoglobin in the resistant corpuscles of 5 cb. mm. of blood.

The accompanying table gives the *relative hæmoglobin-value* of the resistant corpuscles in the blood of some of my students as well in some cases of anæmia in my wards :—

TABLE I.—HEALTH.

(One part of blood + 2 parts of N/50 NaCl solution.)

	Hæmoglobin in 5 cb. mm. of blood.	Hæmoglobin in the resistant corpuscles in 5 cb. mm. of blood.	Relative Hæmoglobin-value of the resistant corpuscles.
(1)	95%	32%	0.336
(2)	96%	40%	0.416
(3)	96%	40%	0.416
(4)	92%	36%	0.391
(5)	98%	35%	0.357
(6)	110%	48%	0.436
(7)	96%	42%	0.437
(8)	92%	34%	0.369

TABLE II.—ANÆMIA.

(1)	40%	8%	0.200
(2)	30%	4%	0.133
(3)	35%	10%	0.285
(4)	36%	10%	0.277

TABLE III.—ANÆMIA.

(1)	60%	21%	0.350
(2)	46%	19%	0.413
(3)	30%	14%	0.467
(4)	36%	15%	0.417

TABLE IV.—ANÆMIA.

(1)	35%	20%	0.571
(2)	42%	23%	0.547

It will be seen from the above tables that while in health the relative hæmoglobin-value of the resistant erythrocytes varies within small limits, in anæmia, on the other hand, wide variations are met with. By this new method of testing the blood we can also divide anæmias into three classes, *viz.* :—(1) those in which this hæmoglobin-value is normal, (2) those in which it is less than normal, (3) those in which it is greater than normal. The following table illustrates the point :—

Case.		Hæmoglobin-value of the Resistant Erythrocytes (1 part of Blood +2 parts of distilled water).
1.	Malaria	.333
2.	„	.606
3.	„	.444
4.	Ankylostomiasis	.714
5.	„	.658
6.	Phthisis	.400
7.	Kala-azar	.266
8.	„	.250
9.	„	.285
10.	„	.142

We thus find that the hæmoglobin-value is markedly diminished in Kala-azar and increased in Ankylostomiasis. Further investigations will, I believe, shew the importance of this method of testing the blood in the differentiation of the various forms of anæmias.

CHAPTER IV.

The freezing point of the resistant corpuscles during hæmolysis. Specific resistance of the erythrocytes to rupture during hæmolysis.

The fact that when blood is treated with distilled water, some of the erythrocytes hæmolyse, while others resist hæmolysis leads to the conclusion that the latter are either less permeable to water or can resist the distension caused by osmosis better than those that hæmolyse. Researches carried on subsequently have shewn that the latter constitutes an important factor in this phenomenon. This leads us to the consideration of *the freezing point of the unhæmolysed corpuscles during hæmolysis of blood.*

In order to determine whether the unhæmolysed corpuscles were less permeable to water or not, the freezing point of the unhæmolysed corpuscles as well as that of the supernatant liquid was estimated, the unhæmolysed corpuscles having been separated by centrifugalisation; the following results were obtained:—

(1)

HUMAN BLOOD.

(*Blood taken from the jugular veins four hours after death.*)

1. Δ for the hæmolysed corpuscles ... .195 C.
2. Δ for the unhæmolysed corpuscles ... .195 C.

(2)

FOWL'S BLOOD.

1. Δ for the hæmolysed corpuscles ... .224 C.
2. Δ for the unhæmolysed corpuscles ... .285 C.

(3)

FOWL'S BLOOD.

1. Δ for the hæmolysed corpuscles ... .210 C.
2. Δ for the unhæmolysed corpuscles ... .210 C.

(4)

FOWL'S BLOOD.

1. Δ for the hæmolysed corpuscles ... .210 C.
2. Δ for the unhæmolysed corpuscles ... .200 C.

(5)

FOWL'S BLOOD.

1. Δ for the hæmolysed corpuscles ... .235 C.
2. Δ for the unhæmolysed corpuscles ... .208 C.

(6)

FOWL'S BLOOD.

1. Δ for the hæmolysed corpuscles ... .230 C.
2. Δ for the unhæmolyted corpuscles ... .190 C.

(7)

1. Δ for the hæmolysed corpuscles ... .250 C.
2. Δ for the unhæmolysed corpuscles ... .250 C.

In the above experiments, the blood was allowed to clot, the clotted blood was squeezed through thin muslin and then mixed with two parts of distilled water, the mixture was then thoroughly centrifuged after one hour.

It will be seen from above that the freezing points of the hæmolysed corpuscles were nearly the same as those of the unhæmolysed corpuscles and, therefore, osmosis must have taken

place to the same extent in the hæmolysed as in the unhæmolysed corpuscles. In other words, the unhæmolysed corpuscles are nearly as much permeable to water as the hæmolysed. The fact that some of the corpuscles do not hæmolyse must, therefore, be due to their being able to resist distension better than those that hæmolyse. We may describe this resistance of the erythrocytes to rupture as their *specific resistance*. This may be expressed in terms of the relative hæmoglobin-value of the resistant erythrocytes, as described in the last chapter. This varies in the case of different animals, as the following table will shew.

In the following table the erythrocytes were invariably suspended in ·85% saline and the amount of suspension taken was always half that of the dissolving fluid, which, in the present case, was distilled water.

SPECIFIC RESISTANCE OF ERYTHROCYTES TO RUPTURE IN DIFFERENT ANIMALS.

1.	Human blood	.2934
2.	Fowl's blood	.1875
3.	Dog's blood	.3333
4.	Frog's blood	.7917
5.	Sheep's blood	0
6.	Rabbit's blood	0

It will be seen from the above table that the erythrocytes of different animals vary in their specific resistance to rupture, the erythrocytes of the sheep and the rabbit being least resistant, while those of the frog are most resistant. This fact may perhaps be of some medicolegal importance and may be utilised in the differentiation of the erythrocytes of an unknown *fresh* specimen of blood. Its application in practice must, however, be evidently limited.

CHAPTER V.

Behaviour of the erythocytes towards hyperosmotic solutions of NaCl—the curves of hæmolysis with hyperosmotic NaCl solutions—the properties of salted erythrocytes.

Behaviour towards very concentrated NaCl solutions:—

When human blood is mixed with 2 volumes of a saturated NaCl solution in distilled water (which we shall henceforth call $\frac{S}{1}$ NaCl Sol.), the mixture at once becomes turbid. The turbidity is followed within a few minutes by a marked solution of the erythrocytes and the mixture at the same time becomes clear to a great extent. In the rabbit's blood no such clearing up of the mixture takes place within a short time and the fluid remains turbid for a much longer time. If the rabbit's blood after it has been treated in the above way be centrifugalised within ten minutes, it is found that the supernatant liquid becomes faintly red, shewing that only slight hæmolysis had taken place, contrary to what is found in the case of human blood in which the supernatant fluid is found to be markedly red.

Similarly, if one part of human blood is mixed with two parts of $\frac{S}{2}$*, $\frac{S}{3}$*, up to $\frac{S}{7}$* NaCl Sol. a similar turbidity is noticed, but at first no hæmolysis is observed within a few minutes. If the sediment obtained, after centrifuging each of the above mixtures, be mixed with the supernatant fluid at its top, we find that the sediment dissolves more and more, each time the process is repeated, making the supernatant fluid more and more red; finally a

* $\frac{S}{1}$ or saturated NaCl solution = 6 $\frac{N}{1}$ NaCl nearly at the temperature at which I have worked; $\frac{S}{2}$ = one part of a saturated NaCl solution diluted with one part of distilled water; $\frac{S}{3}$ = one part of a saturated NaCl solution diluted with 2 parts of distilled water, &c. &c.

sediment remains behind which does not dissolve any more and which is smaller in amount the greater the strength of the NaCl solution. With $\frac{8}{33}$ we find generally no hæmolysis takes place.

The sediment from each of the above mixtures also possesses the remarkable property of dissolving in $\frac{N}{10}$ NaCl solution, the amount dissolved varying with the strength of the NaCl with which the blood was previously treated. Thus the sediment from the mixture containing $\frac{8}{1}$ NaCl dissolves completely, while that containing $\frac{8}{7}$ dissolves only partially in the $\frac{N}{10}$ NaCl solution. It is thus seen that while $\frac{N}{10}$ NaCl acts as an *inactive* fluid towards the sediment of a mixture of blood and two vols. of a hyposmotic NaCl solution, it has the property of dissolving completely the sediment from blood which has been previously treated with two vols. of $\frac{8}{1}$ NaCl solution.

The amount of hæmolysis obtained by mixing blood with the concentrated NaCl solution increases with the extent of time the blood is kept mixed with the solution. Thus, when human blood is mixed with $\frac{8}{7}$ NaCl solution, the supernatant fluid at first appears colourless, but after an hour some hæmolysis is observed in the mixture.

From the above it will be seen that in describing the curve of hæmolysis in human blood treated with hyperosmotic NaCl solutions, we have to take the following factors into consideration :—(1) Effect of time. (2) Effect of repeated centrifugalisations. In this way three curves can be described :—(1) Curve of hæmolysis in human blood treated with varying dilutions of $\frac{8}{1}$ NaCl solution and the mixture very quickly centrifugalised.

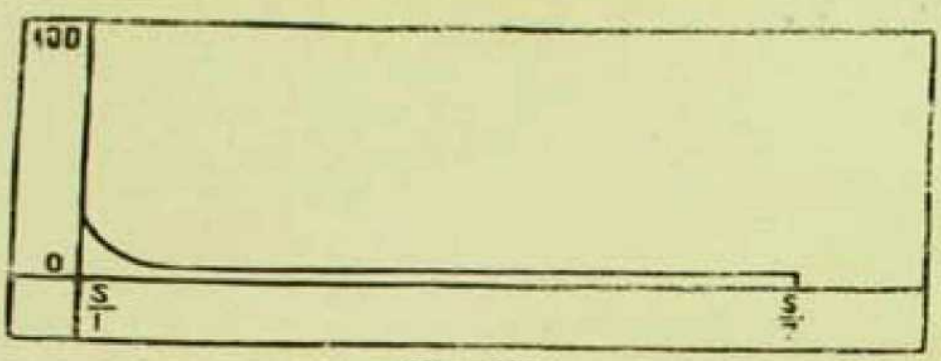


Fig. II

Curve of hæmolysis with hyper-osmotic NaCl solution, the mixture being very quickly centrifuged (one part of human blood + two parts of NaCl solution of strengths between $\frac{S}{1}$ and $\frac{S}{7}$)

(Page 19)

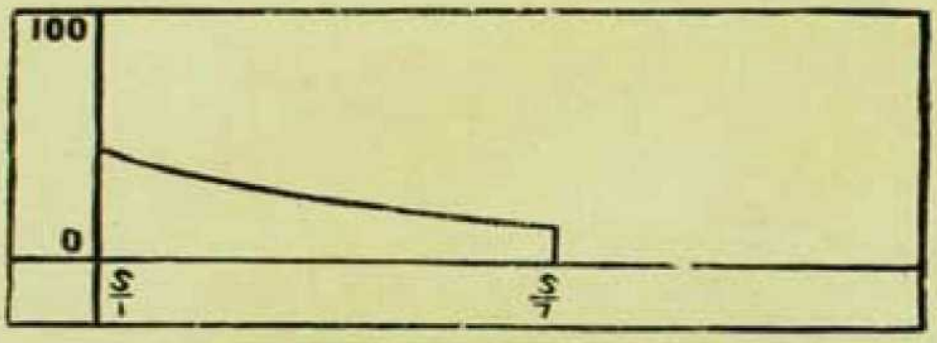


Fig. III

Curve of hæmolysis with hyper-osmotic NaCl solution, after repeated centrifugalisation of the mixture (one part of blood + two parts of NaCl solution of strengths between $\frac{S}{1}$ and $\frac{S}{7}$)

(Page 19)

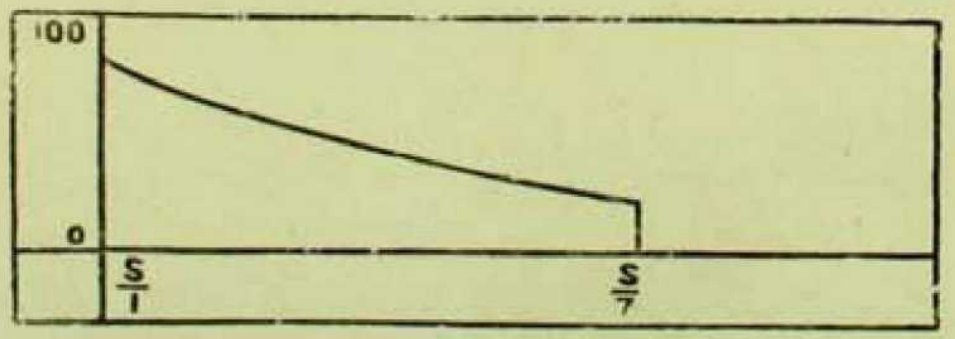


Fig. IV

Curve of hæmolysis with hyper-osmotic NaCl solution, one hour after mixture of the blood with the NaCl solution (one part of blood + two parts of NaCl solution of strengths between $\frac{S}{1}$ and $\frac{S}{7}$)

(Page 19)

(2) Curve of hæmolysis after repeated centrifugalisations. (3) Curve of hæmolysis in the mixture after sometime (say an hour). These are represented in Figs. II, III and IV.

The salted erythrocytes:—By salted erythrocytes, I mean, the the erythrocytes that are found in the sediment after centrifugalisation of a mixture of blood and a very hyperosmotic NaCl solution.

The behaviour of human blood towards saturated NaCl solution has been already described. We now proceed to describe the properties of the salted erythrocytes. As the erythrocytes of the rabbit's blood shew these properties very distinctly and as they have not been studied by any previous observer, I shall describe them here at some length. The erythrocytes of human blood also possess the same properties to a more or less extent.

(1) If the sediment of the rabbit's blood obtained in the above way be mixed with the supernatant fluid at its top, it possesses the remarkable property of dissolving to some extent, shewing, as it were, the hæmoglobin was squeezed out of the erythrocytes during the process of centrifugalisation (see Figs. V and VI). This phenomenon was discovered in the following way:—rabbit's blood was mixed with 2 volumes of a saturated NaCl solution in two separate tubes; one of the mixtures was then centrifugalised and the other one left undisturbed. The sediment in the centrifugalised blood was mixed with its supernatant fluid and the mixture was again centrifugalised and so on. It was found that each time that the sediment was mixed with the supernatant fluid after each successive centrifugalisation and then the mixture centrifugalised again, the tint of the supernatant fluid become more and more

red. On the other hand, when the mixture in the other tube was centrifugalised after quarter of an hour during which the former experiment was performed, it shewed very much less red tint at the top than in the supernatant fluid in the other tube. From this it is evident that the salted erythrocytes undergo hæmolysis each time they are centrifugalised, the hæmoglobin being, as it were, squeezed out of them in their vibration during centrifugalisation and also when they are jammed against each other at the bottom of the centrifugalising tube.

(2) The corpuscles in the same sediment dissolve to a very great extent when treated with an $\frac{N}{10}$ NaCl solution.

(3) The salted erythrocytes are found to be contracted in size, but many of them present a globular shape and do not appear wrinkled or crenated, as one would expect to observe from the membrane-theory of the walls of the erythrocytes. Some of them still contain hæmoglobin, while others are decolourised. Many of those that are decolourised present a globular appearance under the microscope (see plate III). That they are contracted is shewn by the following table :—

	Volume of normal red corpuscles to total blood.	Volume of the salted red corpuscles to total blood. (Blood + 2 vols. of $\frac{S}{2}$ NaCl.)
Rabbit	$\frac{51}{100}=0{\cdot}51$	$\frac{28}{100}=0{\cdot}28$
Human	$\frac{51}{100}=0{\cdot}51$	$\frac{30}{100}=0{\cdot}30$
Human	$\frac{52}{100}=0{\cdot}52$	$\frac{42}{100}=0{\cdot}42$

The method by which I estimated the volume of the

BCU 1846

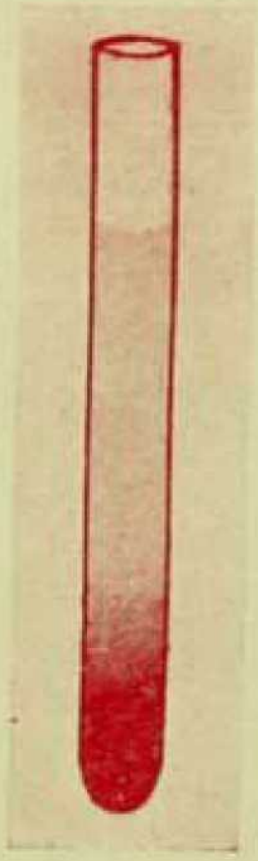


FIG. V.

FIG. VI.

Fig. V.—The supernatant fluid obtained by centrifuging a mixture of one part of rabbit's blood and two parts of saturated NaCl solution, the centrifugalisation being made within a quarter of an hour after the mixture of the blood with the NaCl solution—very faint red colour in the supernatant fluid.

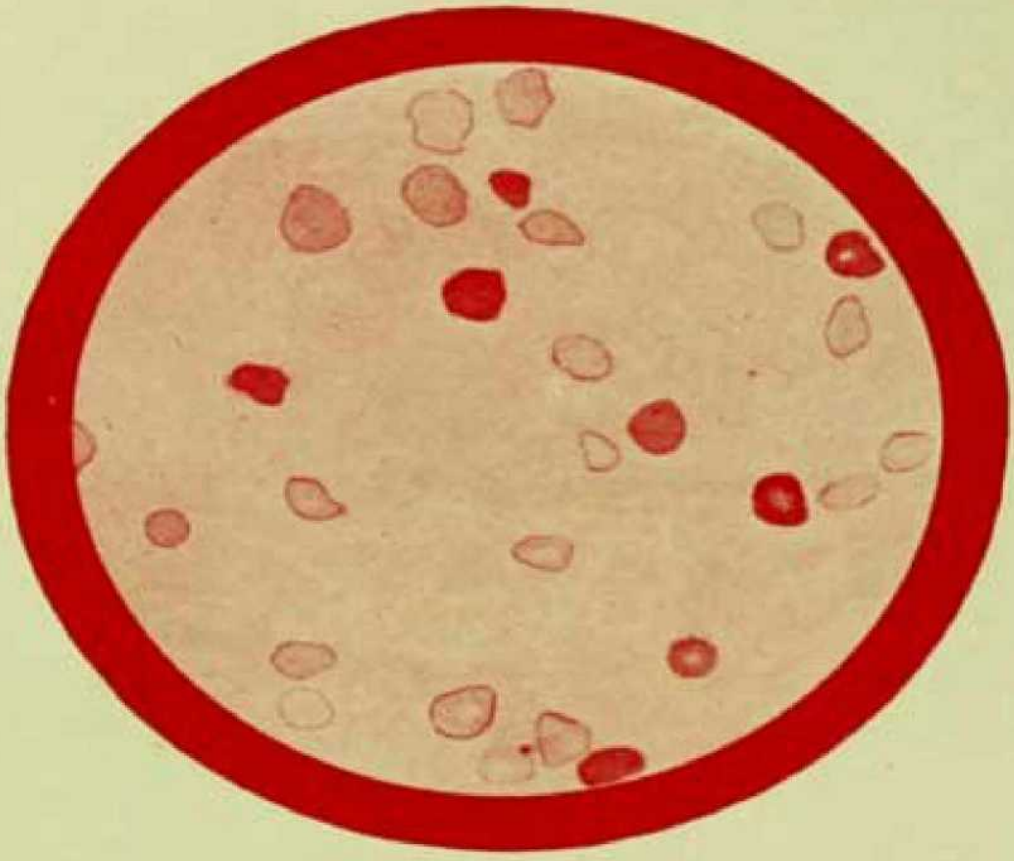
(Page 19.)

Fig. VI.—The sediment from Fig. V mixed with supernatant fluid at the top—marked redness of the supernatant fluid after centrifugalisation.

(Page 19.)

CENTRAL LIBRARY

PLATE III.



The salted erythrocytes of the rabbit obtained by centrifuging a mixture of one part of blood and two parts of saturated NaCl solution.

(Page 20.)

corpuscles in normal blood consisted in mixing a specimen of the blood with a definite volume of 2½% solution of $K_2Cr_2O_7$, and calculating from this the volume of the corpuscles after centrifugalisation. In the case of the blood treated with $\frac{S}{1}$ or $\frac{S}{2}$ NaCl, the mixture was simply centrifugalised and since no coagulation took place, the volume was easily calculated.

I would now summarise all the phenomena presented by the rabbit's blood when treated with a saturated NaCl solution :—

(1) Rabbit's blood + $\frac{S}{1}$ NaCl = Red sediment on centrifugalisation, and at first a colourless supernatant fluid at the top.

(2) The sediment from (1) + the supernatant fluid = some solution of the erythrocytes ; on repeated centrifuging and mixing the sediment with the supernatant fluid, more and more of the erythrocytes dissolve in it.

(3) Sediment from (1) + $\frac{N}{15}$ NaCl solution = very marked solution of the erythrocytes.

(4) Sediment from (1) shews erythrocytes much contracted in size, but many of them present a globular shape and are not wrinkled or crenated ; some contain hæmoglobin, others are decolourized.

(5) Blood + $\frac{S}{1}$ NaCl solution = a very turbid appearance ; this turbidity lasts longer in the case of the rabbit's than in the case of human blood ; at first the turbid fluid when centrifugalised shews no red tint in the supernatant fluid at the top in the case of the rabbit's blood, but in the case of man, a red tint always appears. After an hour, the mixture of the rabbit's blood also shews a red tint in the supernatant fluid after centrifugalisation.

GS 2372

The most probable explanation of the above phenomena appears to me to be a marked change in the outer wall of the erythrocytes brought about by the NaCl of the saturated solution; probably a sort of combination takes place between the NaCl and the outer walls of the erythrocytes. In the next chapter, I shall shew the possibility of the presence of such a compound. This compound finally leads to the destruction of some portion of the walls of the erythrocytes.

When blood is mixed with saturated NaCl solution, no doubt water comes out of the erythrocytes by the process of osmosis and they accordingly contract; when the sediment from the above is treated with $\frac{N}{10}$ NaCl, water re-enters their structure and as a result of this, they try to expand and regain their original size. But either they burst before or as soon as they recover their original size or it may be that the water of the $\frac{N}{10}$ NaCl solution decomposes or dissolves the compound of the NaCl and the outer wall of the erythrocytes. This compound is probably very easily decomposed.

It is evident that *Osmosis* alone can not explain the hæmolysis of blood by saturated NaCl solution. The remarkable phenomenon of hæmoglobin coming out of the corpuscles during centrifugalisation is probably explained by assuming that the damaged walls of the erythrocytes allow hæmoglobin to pass through them by a process allied to *Filtration* under high pressure. As soon as there is decomposition of the unstable compound of the NaCl with the outer wall of the erythrocytes, the latter behave like small spheres of sponges containing dissolved colouring matter. Probably the same changes also

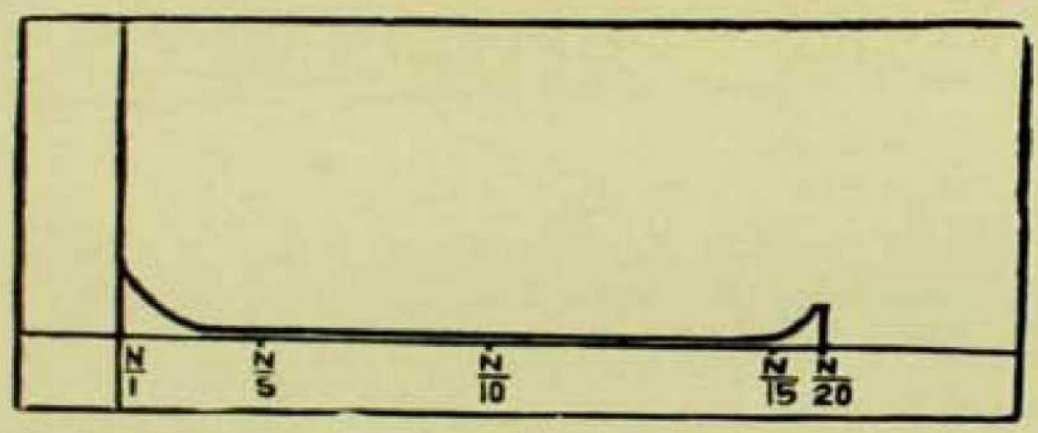


Fig. VII.—Curve of hæmolysis with NaCl solution of strengths between $\frac{N}{1}$ and $\frac{N}{20}$ (one part of human blood + two parts of NaCl solution).

(Page 23.)

On page 23 in line 15 *read* Fig. VII *for* Fig. VI.

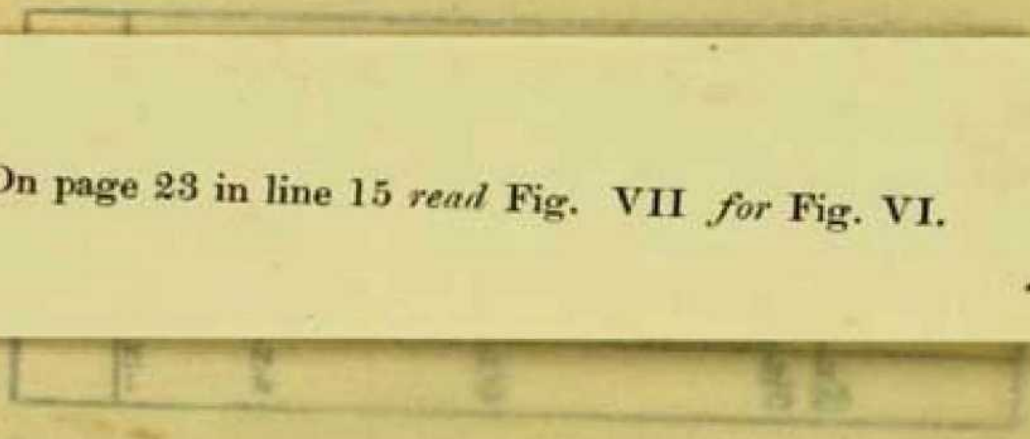


Fig. VII.—Curve of hæmolysis with NaCl solution of strengths between $\frac{N}{1}$ and $\frac{N}{25}$ (one part of human blood + two parts of NaCl solution).

take place in the erythrocytes when blood is treated with $\frac{8}{3}$ or half-saturated NaCl solution.

Behaviour of the erythrocytes towards less concentrated solutions of NaCl :—

When human blood is treated with two volumes of a hypertonic NaCl solution of the strength $\frac{8}{7}$, we find that the mixture becomes somewhat opaque and bright red. As stated before, the sediment from such a mixture when mixed with the supernatant fluid at the top shews some hæmolysis, but it is much less than when the blood is treated with $\frac{8}{4}$ NaCl solution.

Behaviour of blood towards NaCl solutions of strengths between $\frac{8}{7}$ and $\frac{N}{20}$ NaCl solution :—*

Between these limits we find that with $\frac{N}{2}$, generally no hæmolysis takes place, so also with $\frac{N}{10}$ and $\frac{N}{15}$ NaCl solutions. See Fig. VI.

---

* According to my calculation, $\frac{8}{7}$ NaCl = $\frac{2}{7}$ $\frac{N}{1}$ NaCl nearly.

CHAPTER VI.

Possibility of absorption of NaCl by the erythrocytes when blood is treated with saturated or half-saturated NaCl solution.

The researches of Hedin[1], Eykman[2], Stewart[3], and others shew that the permeability of the erythocytes for NaCl is very slight or none at all. As far, however, as I am aware there are no records of any observations of the action of saturated or half-saturated NaCl solution upon erythrocytes.

I have shewn in the former chapter that the properties of salted erythrocytes would lead one to the supposition that a possible combination takes place between the NaCl of the saturated or half-saturated NaCl solution and the erythrocytes. Whether any NaCl is absorbed or not by the erythrocytes when blood is treated with saturated or half-saturatad NaCl solution can be determined in two ways :—

(1) By taking the electric conductivity of the blood before and after the mixture.

(2) By actual chemical analysis of the supernatant fluid after centrifugalisation of the mixture.

I have made some observations according to the second method of investigation and the few experiments, that I have made, shew the great possibility of the absorption of NaCl by the erythrocytes under the circumstances mentioned above.

---

[1] Pflüger's Archives LXVIII, 1897.

[2] Ibid S. 58.

[3] Journal of Physiology, Vol. XXVI, 1901.

As this method of investigation is my own, I describe it in detail :—

This *chemical analysis method* depends upon the following calculations :

(1) Volume of the serum in normal blood.

(2) Volume of the serum in blood treated with $\frac{8}{1}$ or $\frac{8}{2}$ NaCl solution.

(3) The quantitative estimation of the chlorides of the serum.

(4) The quantitative estimation of NaCl in the solution of NaCl used, say $\frac{8}{2}$.

(5) The quantitative estimation of the chlorides in the serum after blood is mixed with, say, two volumes of $\frac{8}{2}$ NaCl solution.

(6) The theoretical estimation of the chlorides in the above serum.

(7) The presence of any difference between the above two estimations *i.e.*(5) and (6) and its explanation.

1st experiment.

Rabbit,

(1) One part of rabbit's blood was taken and centrifugalised after being mixed with $2\frac{1}{2}\%$ solution of $K_2Cr_2O_7$. It was found by an average of 3 experiments :

$$\frac{\text{Volume of Serum}}{\text{Volume of Blood}} = \frac{51}{100} \text{ or } \frac{1}{2} \text{ nearly.} \quad (1)$$

(2) One volume of blood was mixed with 2 vols. of $\frac{8}{2}$ NaCl solution and the mixture was quickly centrifugalised. It was found that $3\frac{3}{4}$ parts of the mixture contained $3\frac{1}{16}$ parts of

supernatant fluid *i.e.*, $\frac{49}{54}$ part of the supernatant fluid or diluted serum was contained in one part of the mixture. (2)

(3) By quantitative estimation, the chlorides of the rabbit's serum were found as follows :—

1 vol. of serum $= \frac{3}{250}$ vol. of $\frac{N}{1}$ NaCl. (3)

(4) The quantitative estimation of the chlorides in the supernatant fluid after centrifugalisation of a mixture of 1 part of blood and 2 parts of $\frac{8}{9}$ NaCl solution was found to be, by an average of three experiments, as follows :—

1 vol. of the supernatant fluid = 1·833 vol. of $\frac{N}{1}$ NaCl. (4)

(5) By actual experiment it was also found

1 vol. of $\frac{8}{9} = 1$ vol. of $\frac{60}{250}$ $\frac{N}{1}$ NaCl or 3 vol. of $\frac{N}{1}$ NaCl. (5)

It is evidently from the above we should have in the serum obtained from a mixture of and 1 volume of blood, 2 vols. of $\frac{8}{9}$ NaCl an amount of NaCl which is contained in $(6 + \frac{1}{2} \times \frac{3}{250})$ vol. of $\frac{N}{1}$ NaCl, *provided no combination has taken place between the corpuscles and the NaCl of the* $\frac{8}{9}$ *NaCl solution.*

Also 3 volumes of the mixture contain $3 \times \frac{49}{54}$ or $\frac{49}{18}$ volumes of supernatant fluid.

$\therefore$ $\frac{49}{18}$ volumes of the supernatant fluid contain the amount of NaCl which is present in $\frac{303}{500}$ volumes of $\frac{N}{1}$ NaCl.

$\therefore$ 1 volume should contain an amount of NaCl that is present in $\frac{303}{500} \times \frac{18}{49}$ *i.e.* 2·23 volumes of $\frac{N}{1}$ NaCl. (6)

But by actual experiment it was found that 1 volume of the supernatant fluid contained an amount of NaCl which is present in 1·833 volumes of $\frac{N}{1}$ NaCl.

$\therefore$ The amount of NaCl contained in ·397 volume of $\frac{N}{1}$ NaCl solution must have been absorbed by the erythrocytes of the blood.

2nd experiment :

Student,

(1) In estimating the volume of the serum compared with that of the blood, the same method as in the former case was adopted.

∴ It was found from this 1 volume of blood contained $\frac{45}{88}$ or nearly $\frac{1}{2}$ volume of pure serum. (1)

(2) One volume of blood was mixed with 2 volumes of $\frac{8}{3}$ NaCl solution and the mixture was quickly centrifugalised. It was found that $\frac{5}{4}$ volumes of the mixture contained $\frac{9}{8}$ volumes of the supernatant fluid.

∴ $\frac{9}{10}$ volume of the supernatant fluid was contained in one volume of the mixture. (2)

(3) By quantitative estimation, the chlorides of this student's serum were found to be as follows :—

1 volume of serum = $\frac{3}{25}$ vol. of $\frac{N}{1}$ NaCl. (3)

(4) The quantitative estimation of the chlorides in the supernatant fluid after centrifugalisation of the mixture of 1 part of blood and 2 parts of $\frac{8}{3}$ NaCl solution was found to be as follows :—

1 volume of the supernant fluid = 2 volumes of $\frac{N}{1}$ NaCl. (4)

(5) By actual calculation it was found

1 vol. of $\frac{8}{3}$ = 3 volumes of $\frac{N}{1}$ NaCl. (5)

From the above we should have in the supernatant fluid, obtained from a mixture of 2 volumes of $\frac{8}{3}$ NaCl and 1 volume of blood, an amount of NaCl which is contained in $(6 + \frac{1}{2} \times \frac{3}{25})$ volumes of $\frac{N}{1}$ NaCl, *provided that no combination has taken place between the corpuscles and the NaCl of the* $\frac{8}{3}$ *solution.*

Also 3 volumes of the mixture contain $3 \times \frac{9}{10}$ or $\frac{27}{10}$ volumes of the supernatant fluid.

∴ $\frac{27}{10}$ volumes of the supernatant fluid contain an amount of NaCl which is present in $\frac{303}{500}$ volumes of $\frac{N}{1}$ NaCl.

∵ 1 volume contains an amount of NaCl which is present in $\frac{303}{500} \times \frac{10}{27}$ or 2·24 volumes of $\frac{N}{1}$ NaCl. (6)

But by actual experiment, 1 volume of the supernatant fluid contained an amount of NaCl which is present in 2 volumes of $\frac{N}{1}$ NaCl solution.

∴ the amount of NaCl which is contained in ·24 volumes of $\frac{N}{1}$ NaCl solution must have been absorbed by the erythrocytes.

3rd experiment :

Student,

(1) Here too in estimating the volume of the serum compared with that of blood, the same method as in the above experiments was adopted.

It was found that one volume of blood contained $\frac{14}{29}$ or nearly $\frac{1}{2}$ vol. of pure serum. (1)

(2) One volume of blood was mixed with 2 vols. of $\frac{8}{9}$ NaCl solution and the mixture was quickly centrifugalised.

It was found $\frac{51}{86}$ volumes of the supernatant fluid was contained in one volume of the mixture. (2)

(3) By quantitative estimation, the chlorides of the student's serum was found as follows :—

1 volume of serum $= \frac{11}{100} \frac{N}{1}$ NaCl. (3)

(4) The quantitative estimation of the chlorides in the supernatant fluid after centrifugalisation of the mixture of 1 part of blood and 2 parts of $\frac{8}{9}$ NaCl solution was found to be as follows :

1 volume of the supernatant fluid = 2 volumes of $\frac{N}{1}$ NaCl (4)

(5) By actual experiment it was found that 1 volume of $\frac{S}{2}$=3 of $\frac{N}{1}$ NaCl. (5)

From the above we should have in the supernatant fluid obtained from a mixture of 2 volumes of $\frac{S}{2}$ NaCl and 1 volume of blood, an amount of NaCl which is present in $(6 + \frac{1}{2} \times \frac{11}{100})$ volumes of $\frac{N}{1}$ NaCl, *provided that no combination has taken place between the corpuscles and the NaCl of the* $\frac{S}{2}$ *solution.*

Also 3 volumes of the mixture contain $\frac{311}{112}$ volumes of the supernatant fluid.

$\therefore$ $\frac{311}{112}$ volumes of this fluid contain an amount of NaCl which is present in $\frac{1211}{200}$ volumes of $\frac{}{1}$ NaCl.

$\therefore$ 1 volume contains an amount of NaCl which is present in $\frac{1211}{200} \times \frac{112}{311}$ or 2·34 volumes of $\frac{N}{1}$ NaCl solution. (6)

But by actual experiment it was found that one volume of the supernatant fluid contained an amount of NaCl which is present in 2 volumes of $\frac{N}{}$ NaCl.

$\therefore$ the amount of NaCl which is contained in ·34 volumes of $\frac{N}{1}$ NaCl solution must have been absorbed by the erythrocytes.

The above method was also adopted to determine whether any combination takes place between the erythrocytes of the rabbit's blood and $\frac{}{1}$ NaCl, when the former is mixed with two volumes of $\frac{N}{1}$ NaCl solution. The following data were thus obtained :—

(1) Volume of serum $=\frac{11}{100}$ volume of the blood or nearly $\frac{1}{2}$.

(2) By centrifugalisation of a mixture of one part of blood and two parts of $\frac{N}{1}$ NaCl solution, it was found that the volume of the corpuscles was $\frac{3}{5}$ths that of the blood.

$\therefore$ 2 volumes of $\frac{N}{1}$ NaCl + 1 volume of blood contain $2\frac{3}{5}$ volumes of the diluted serum.

By actual calculation it was found that one volume of serum $= \frac{1}{2\frac{3}{5}} \frac{N}{1}$ NaCl.

$\therefore$ $2\frac{3}{5}$ volumes of the diluted serum contain an amount of NaCl which is present in 2 volumes of $\frac{N}{1}$ NaCl + 1 vol. of $\frac{1}{100} \times \frac{3}{2\frac{3}{5}} \frac{N}{1}$ NaCl, *provided that no combination has taken place between the corpuscles and the NaCl of the* $\frac{N}{1}$ *NaCl solution.*

i.e., $2\frac{3}{5}$ volumes of the diluted serum $= 2{\cdot}062$ vols. of $\frac{N}{1}$ NaCl or 1 vol. $= \frac{1}{100} \frac{3}{2\frac{3}{5}}$ vol. of $\frac{N}{1}$ NaCl $= {\cdot}7931$ vol. of $\frac{N}{1}$ NaCl.

By actual calculation it was found that

2 vols. of the diluted serum $= 4 \times 4 \times \frac{1}{10}$ vol. of $\frac{N}{1}$ Ag NO_3

or one volume $= {\cdot}8$ vol. of $\frac{N}{1}$ NaCl.

$\therefore$ The difference between the two calculations is $({\cdot}8 - {\cdot}7931)$ vol. of $\frac{N}{1}$ NaCl $= {\cdot}0069$ vol. of $\frac{N}{1}$ NaCl, which is within the limits of errors of observation. Therefore, no combination takes place between the erythrocytes of the rabbit's blood and the NaCl of the $\frac{N}{1}$ NaCl and this is also confirmed by the fact that hardly any hæmolysis takes place when 1 volume of the rabbit's blood is mixed with 2 vols. of $\frac{N}{1}$ NaCl solution, contrary to what is found when one volume of the rabbit's blood is mixed with 2 vols. of $\frac{8}{1}$ or $\frac{8}{2}$ NaCl solution.

These few experiments lead to very important results which have not been observed before. If confirmed, they would definitely prove that when blood is treated with very concentrated NaCl solutions, the NaCl enters into combination with the erythrocytes and afterwards destroys their resisting power to hæmolysis. This combination is probably of the nature of *adsorption*.

It is thus seen that my observations agree with those of previous observers in so far that the erythrocytes are generally

impermeable to NaCl when treated with certain concentrations of it, but when treated with very concentrated solutions, such as, saturated or half-saturated solution, combination probably does take place and in this latter respect the behaviour of the erythrocytes is different from what takes place when they are treated with comparatively dilute NaCl solutions. When such combination takes place, the erythrocytes are markedly changed in their character and properties, as compared with those that are normal. As a result of this, the erythrocytes, for a short time, as pointed out in detail previously, behave like spheres of sponges holding dissolved colouring matter. But, sooner or later, a portion of the outer part of the erythrocytes is separated from its other constituents and as a result of this there is marked hæmolysis as well as complete disintegration of the erythrocytes, as is shewn by the fact that shadow corpuscles are fewer, when blood is treated with $\frac{S}{1}$ or $\frac{S}{2}$ NaCl solution, than when treated with a hyposmotic NaCl solution. This portion of the erythrocytes is perhaps the cell-globulin of Halliburton and Friend,[1] which was afterwards found to be a nucleo-proteid.[2] It has been stated that the stroma of the erythrocytes contains only a small amount of this substance,[3] but it appears to me to be a very important constituent of the erythrocytes preventing the solution of the erythrocytes in normal blood. It probably exists in combination with the lecithin and cholesterin existing in the walls of the erythrocytes.[4]

---

[1] Journal of Physiology, Cambridge and London, 1886, vol. X.

[2] Halliburton, Journal of Physiology, Cambridge and London, 1895, vol. XVIII.

[3] Schafer, Quain's Anatomy, 1901.

[4] Schafer's Physiology, vol. I, 1898.

CHAPTER VII.

Resistance of the erythrocytes to hæmolysis under certain abnormal conditions—Increased resistance of the walls of the erythrocytes under certain abnormal conditions—Hæmalkalinity and Hæm-salinity.

I have already observed that if one volume of normal blood is mixed with two volumes of $\frac{N}{20}$ NaCl solution, slight hæmolysis is not infrequently observed, while with $\frac{N}{20}$ it is often distinct or sometimes even marked. In certain forms of anæmia, 2 volumes of $\frac{N}{20}$ NaCl causes no hæmolysis, while $\frac{N}{30}$ or even $\frac{N}{40}$ NaCl causes very slight or no hæmolysis. In other words, the erythrocytes in some forms of anæmia resist hæmolysis more than normal blood. Major McCay, by estimating the hæmosozoic value of serum in certain forms of anæmia, also arrives at the same conclusion and he thinks that this might be due to the presence of an antihæmolysin[1]. This resistance of the erythrocytes to hæmolysis has been already described by me as their specific resistance. In a case of Black-water fever this specific resistance was found to be so high as ·585 immediately after the attack was over, while five days after the attack it was so low as ·294. It is perhaps increased when blood is treated with a mild solution of formol. Thus it was found that when one part of blood is mixed with one part of $\frac{N}{10}$ saline solution and then treated with two parts of $\frac{N}{20}$ saline, the amount of hæmolysis is much greater than when the $\frac{N}{10}$ saline contains 1 per cent. formol.

---

[1] McCay, Bio-Chemical Journal, Vol. III, 1908.

The resistance of the erythrocytes to hæmolysis that is observed in some forms of anæmia led me to investigate if this could be due to any peculiarities in the serum. It was, therefore, found necessary to determine the *hæm-alkalinity* and *hæm-salinity* of these cases and compare them with normal.

Hæmalkalinity: Tho method adopted was a modification of that described by Moore and Wilson.[1] In this method not more than 10 cb. mm of serum were required and 50 cb. mm of blood enabled me to estimate both hæm-alkalinity and hæm-salinity. The methods devised by Landois[2], Liebreich[3], Haycraft and Williamson[4], and Kraus,[5] for estimating the alkalinity of blood are not, as pointed out by Da Costa,[6] well adapted to routine blood work.

50 cb. mm of blood were taken from the finger, which was sterilized with 5% formol solution, and put into a perfectly dry sterilized tube and then quickly centrifugalised. The tube was then closed air-tight to prevent any evaporation and allowed to stand for some hours and again centrifugalised, so as to free the serum from all the erythrocytes. 10 cb. mm of the serum were treated with a solution of $\frac{N}{100}$ H_2 S O_4 in a white porcelain shallow capsule, using a drop or two of a fresh dilution of an alcoholic solution of Di-methyl-amido-azo-benzol in distilled water as an indicator,* the first begining of neutralisation being

---

[1] Moore and Wilson, Bio-Chemical Journal, Vol. I, 1906.

[2] Real-Encyclop, 1885, Vol. III.

[3] Berichte d. deutsch, chem. Gessellsch, 1868.

[4] Proceedings, Royal Society, Edinburgh, 1888.

[5] Zeitschr. F. Heilk., 1889.

[6] Da Costa, Clinical Hæmatology.

* I generally mix one drop of the alcoholic solution with about 2 cc. of distilled water just at the time of the experiment. A few drops of this mixture are added to the serum to make it faintly yellow.

indicated by a faint rose colour at the side of the solution in the porcelain capsule.

Hæm-salinity :—The salinity of the blood was estimated by treating 10 cb. mm of blood with $\frac{N}{100}$ $AgNO_3$ using a solution of $K_2Cr\,O_4$ as an indicator.

A series of observations were made on the blood of healthy studeuts as well as of some cases of anæmia and the results obtained are appended in the accompanying tables.

Salinity and Basic Reactivity of normal blood.

		Salinity.	Basic Reactivity.	Commencing hæmolysis was brought about by 2 vols. of
1	N. N. A.	·6435%	·200 Normal	$\frac{N}{20}$ NaCl.
2	D. N. R.	·5850%	·180 „	Do.
3	R.	·6435%	·160 „	Do.
4	S.	·7020%	·160 „	Do.
5	H. N. S.	·7020%	·210 „	Do.
6	I.	·6435%	·200 „	Do.
7	G.	·7020%	·170 „	Do.
8	S. C. R.	·7020%	·150 „	Do.
	Average.	=·6654%	=·178 $\frac{N}{1}$	Do.

Salinity and Basic Reactivity of the serum in some forms of anæmia (Ankylostomiasis.)

		Salinity.	Basic Reactivity.	Red corpuscle and Hæmoglobin.	Disease.	Commencing hæmolysis was brought about by 2 Vols. of
1	Patu	·7605%	·140 Normal	R.B.C. = 1686000 Hb = 15%	Ankylostomiasis (no œdema)	$\frac{N}{10}$ NaCl
2	Hirso	·5850%	·095 „	R.B.C. = 1770000 Hb = 13%	Do.	$\frac{N}{10}$ Do.
3	Panchu ...	·6727%	·140 „	R.B.C. = 2600000 Hb = 60%	...	$\frac{N}{10}$ Do.
4	... ...	·6724%	·0675 „	Hb = 5%	Ankylostomiasis (Very marked œdema)	$\frac{N}{10}$ Do.
	Average ...	·6668%	·1185 „			

Salinity and Basic Reactivity of the serum in some forms of anæmia (not Ankylostomiasis.)

1	Pryag	·6435%	·150 Normal	R.B.C. = 1920000 Hb = 40%		
2	Bhoirab ...	·8772%	·110 „	R.B.C. = 2180000	Cancer of the Stomach.	$\frac{N}{10}$ NaCl

It will be seen from the above that while there was not much appreciable difference in the chlorides of the *serum* in some of the cases of anæmia compared with the normal, there was rather a distinct diminution in the alkalinity of the *serum* in almost all of them. This latter fact, so far as I am aware, has not been noted by any previous observer. V. Jaksch, however, by titration of *opaque blood* also finds diminution of alkalinity in all anæmias.[1]

It is evident that the resistance of the erythrocytes to hæmolysis in some cases of anæmia (especially ankylostomiasis) is not due to the presence of any excess of chlorides in the serum. Besides, in some of these cases, I washed the erythrocytes several times with a deci-normal solution of NaCl, till the supernatant fluid obtained on centrifugalisation was found to be perfectly free from the slightest trace of albumen. One volume of the suspension of the erythrocytes was now treated with 2 vols. of $\frac{N}{10}$ NaCl and it was found that the resulting mixture did not shew any hæmolysis at all, while normal blood under similar circumstances shewed marked hæmolysis.

The diminition in the alkalinity of the serum in the cases of anæmia cannot, however, explain the increased resistance of the erythrocytes to hæmolysis, because diminished alkalinity increases the tendency to hæmolysis.[2]

The resistance of the erythrocytes to hæmolysis in cases of anæmia is not, therefore, due to anything present in the serum. Whatever may be the cause, it must exist in the red corpuscles themselves.

It may be stated here that there was a distinct increase in the salinity of the serum in the case of cancer of the stomach.

---

[1] V. Jaksch, Zeits F. Klin Med, Vol. XIII, 1887. [2] Schafer's Physiology, Vol. I.

PLATE IV.

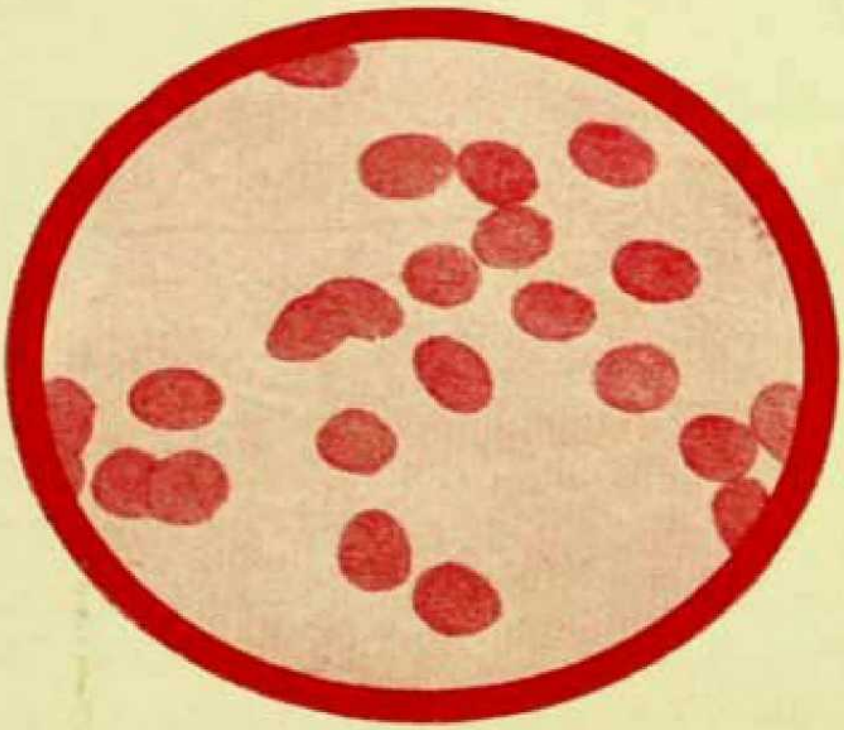


FIG. I.

Fig. I.—Normal erythrocytes of man fixed in absolute alcohol and stained with eosin. (Page 37.)

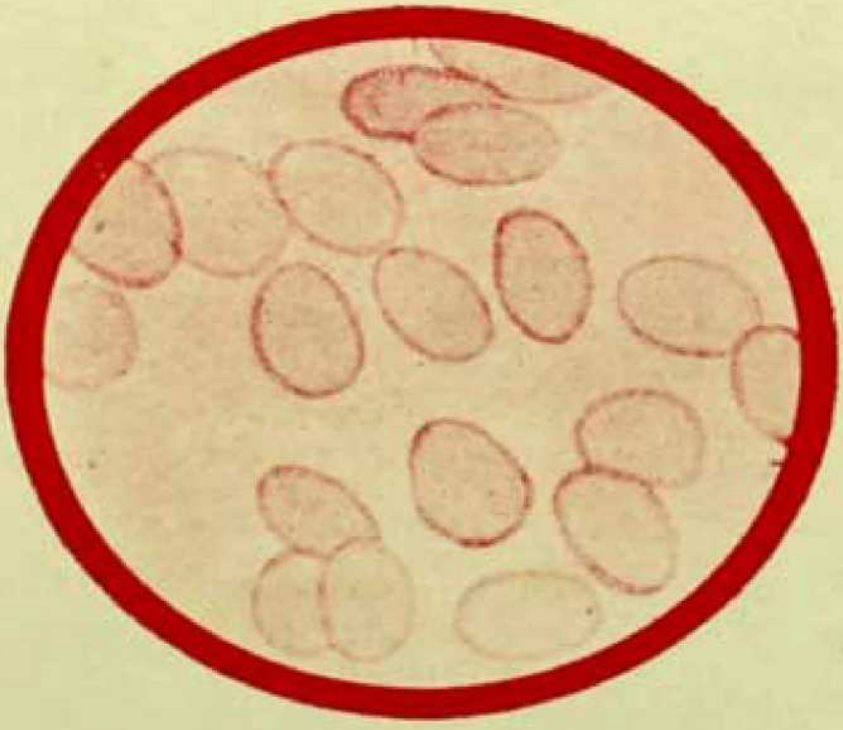


FIG. II.

Fig. II.—Suspension of erythrocytes of man in $\frac{N}{10}$ NaCl solution is gently dropped over a slide and allowed to dry at the temperature of the room (29°C); then they are treated with $\frac{N}{10}$ NaCl—the swollen appearance of the erythrocytes before they dissolve. (Page 37.)

CHAPTER VIII.

The effects of evaporation on the resistance of the erythrocytes to hæmolysis. Crenation of erythrocytes.

If a specimen of blood be washed in an $\frac{N}{10}$ NaCl solution till all the serum is completely removed, and if the suspension of the red corpuscles, that is thus obtained, be gently dropped over a slide and then allowed to dry, the erythrocytes over the slide possess the remarkable property of dissolving in $\frac{N}{10}$ NaCl solution, though the latter has no action on the erythrocytes that have not undergone this sort of drying up.

The erythrocytes before undergoing hæmolysis become markedly swollen in size, as shewn in plate IV. This phenomenon was observed as follows :—

A drop of $\frac{N}{10}$ NaCl solution was put over a cover-glass which was now placed over the dried specimen of blood on the slide, the surface with the drop of $\frac{N}{10}$ NaCl on it being made to come in contact with the erythrocytes on the slide. It was found that the erythrocytes which were present near to the edge of the cover-glass and which had been undergoing hæmolysis became markedly swollen in size evidently due to the absorption of water, while those in the centre had undergone complete solution. An explanation of this phenomenon will be offered in the last chapter.

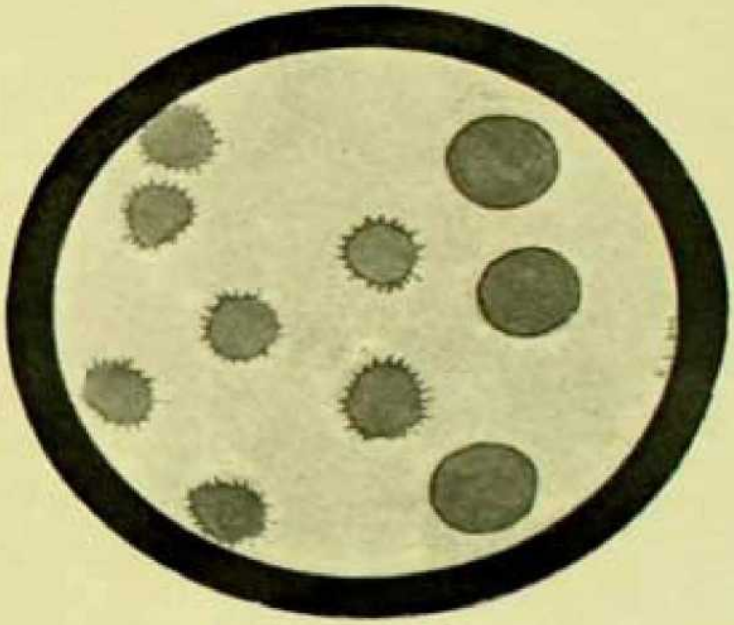
The swollen appearance of the hæmolysing corpuscles makes it probable that they are more permeable to water than normal corpuscles. This leads one to the consideration of the question as to whether the permeability of erythrocytes to water may

be made to vary. The behaviour of crenated corpuscles leads one to think that this may be the case, as is shewn by the following experiment ;—

A drop of blood is placed on a slide and then a coverglass is gently put over it. The blood corpuscles are allowed to crenate for 24 hours. A solution of $\frac{N}{10}$ NaCl is now gently poured over the edge of the coverglass which is very carefully removed. It will be found that many of the erythrocytes dissolve, others look spherical, while there are others which still are crenated (See Fig. VIII).

Now if crenation were simply due to ex-osmosis of water, the corpuscles would swell up and lose their crenation by re-absorption of water when treated with $\frac{N}{10}$ saline solution. The fact that some of them do not lose their crenation shews that they have become less permeable to water. In other words, along with crenation the outer portion of the erythrocytes undergo some changes as a result of which they do not allow the free passage of water into their structure by the process of end-osmosis. The same is also borne out by the fact that crenated corpuscles occur in the blood in some forms of anæmia. This cannot be due to any stronger concentration of saline in the serum, as in anæmia the chlorides, as already pointed out, are not appreciably increased in the serum.

FIG. VIII.



Red corpuscles allowed to crenate between a cover-glass and a slide. The crenated corpuscles subsequently treated with $\frac{N}{10}$ NaCl solution, some look globular, others still remain crenated.

(Page 38)

(Diagrammatic.)

CHAPTER IX.

Resistance of the erythrocytes of blood that has been kept shed for some time to hæmolysis—Spontaneous hæmolysis—Effect of $\frac{N}{10}$ NaCl upon spontaneous hæmolysis—hæmolysis and coagulation—Effect of X-rays upon the resistance of the erythrocytes to hæmolysis.

If blood is kept in a perfectly sterilized tube, evaporation being prevented by carefully sealing the mouth of the tube, we find that the resistance of the erythrocytes to hæmolysis diminishes in course of time.

Thus we had in some cases :—

1. Freshly drawn blood + 2 vols. of $\frac{N}{20}$ NaCl = faint hæmolysis.
2. Blood kept shed for 24 hours + do. = do. do.
3. Blood kept shed for 48 hours + do. = slight hæmolysis
4. Blood kept shed for 80 hours + do. = distinct hæmolysis.

From the above, it will be seen that the erythrocytes began to lose their power of resistance to hæmolysis eighty hours after the blood was kept shed at the temperature of the room which was 29°C. This loss of the resisting power must be explained as a sign of the commencement of the loss of vitality in the erythrocytes. Similary, it has been shewn that mammalian blood kept in the cold in a flask exposed to the influence of ice water, may retain its functional activity for 4 or 5 days, and that, removed from the body for a longer period of time and

then returned to the circulation, the red corpuscles rapidly undergo destruction.[1] In my observations the power of resistance to hæmolysis was distinctly lost eighty hours after the blood was shed. It will be seen that my method of treating blood with $\frac{N}{210}$ NaCl solution and observing the amount of hæmolysis that has taken place in the mixture, is a much simpler way of determining when the erythrocytes are beginning to lose their vital activities than the difficult and complicated method of returning the erythrocytes to the circulation and then watching whether they are undergoing destruction or not.

The erythrocytes undergo spontaneous hæmolysis in blood that has been kept shed for sometime, and this is independent, as has been pointed out by Stewart,[2] of any bacterial infection. I have observed in some of my experiments no spontaneous hæmolysis taking place in the blood even eighty hours after the blood was kept shed in a perfectly sterilized and closed tube at the temperatute of 20°C. In other cases, I found spontaneous hæmolysis starting earlier. The circumstances which enhance or retard this have still to be investigated. One fact that I have noticed in some of my cases is the retarding of spontaneous hæmolysis by the removal of the clot that was formed in the blood and also by the addition of a little sterilized $\frac{N}{10}$ NaCl solution. These conclusions have, however, to be tested by more extended observations. If corroborated, they might lead to the conclusion that probably something of the nature of a hæmolysin is produced by the disintegration of the leucocytes that are entangled in the clot.

---

[1] Landois, Text-Book of Human Physiology.

[2] Stewart, Journal of Physiology, vol. XXIV, 1899.

• *Effect of X-rays upon the resistance of the erythrocytes to hæmolysis :—*

It has been observed that electric shocks passed through blood, if sufficiently strong, cause hæmolysis. The effect of X-rays is, however, different. I have found that blood exposed to X-rays for 20 to 30 minutes does not shew less resistance to hæmolysis than the blood that has not been thus acted upon. Thus I have seen in some of my cases :—

1. Blood, not exposed to X-rays + 2 vols. of $\frac{N}{10}$ NaCl = slight hæmolysis.
2. Blood, exposed for 20 minutes to X-rays + 2 vols. of $\frac{N}{10}$ NaCl = slight hæmolysis.

CHAPTER X.

The law regulating hæmolysis of erythrocytes in hyposmotic saline or distilled water.

In studying the hæmoglobin-value of the resistant erythrocytes, I have obtained results which lead to the conclusion that the amount of hæmoglobin that can be dissolved in a definite volume of hypotonic saline solution or distilled water is proportional to the total amount of hæmoglobin present in the suspended corpuscles.

In the following tables* t denotes the total amount of hæmoglobin in a suspension of erythrocytes, d the amount of dissolved hæmoglobin in it after its treatment with 2 parts of hyposmotic saline or distilled water and p is equal to $\frac{d}{t}$.

TABLE I (a).

NORMAL HUMAN BLOOD.

(1 part of erythrocytes suspended in ·85% NaCl solution + 2 parts of $\frac{N}{10}$ NaCl solution.)

1. t = 110, d = 62 ; p × 10^2 = 56·30
2. t = 100, d = 58 ; p × 10^2 = 58·00
3. t = 90, d = 50 ; p × 10^2 = 55·55
4. t = 77, d = 44 ; p × 10^2 = 57·14

* These tables are extracted from the author's paper in the Bio-Chemical Journal.

TABLE I (b).

Normal Human Blood.

(1 part of erythrocytes suspended in ·85% NaCl + 2 parts of distilled water.)

1. t = 150, d = 106 ; p × 10^2 = 70·66
2. t = 118, d = 90 ; p × 10^2 = 76·27
3. t = 50, d = 35 ; p × 10^2 = 70·00

TABLE II.

Fowl's Blood.

(1 part of erythrocytes suspended in ·85% NaCl solution + 2 parts of distilled water.)

1. t = 105, d = 88 ; p × 10^2 = 83·80
2. t = 96, d = 78 ; p × 10^2 = 81·25
3. t = 90, d = 80 ; p × 10^2 = 88·88
4. t = 84, d = 68 ; p × 10^2 = 80·95
5. t = 42, d = 34 ; p × 10^2 = 80·95

TABLE III (a)

Sheep's Blood.

(1 part of erythrocytes of the sheep suspended in the serum of the sheep + 2 parts of distilled water.)

1. t = 120, d = 120 ; p × 10^2 = 100·00
2. t = 70, d = 70 ; p × 10^2 = 100·00

TABLE III (b).

Sheep's Blood.

(1 part of erythrocytes suspended in ·85% NaCl solution + 2 parts of distilled water.)

1. t = 150, d = 150 ; p × 10^2 = 100·00
2. t = 70, d = 70 ; p × 10^2 = 100·00

TABLE IV (a).

Frog's Blood.

(1 part of erythrocytes suspended in the serum of the frog + 2 parts of $\frac{N}{50}$ NaCl).

1. t = 46, d = 10 ; p × 10^2 = 20·87
2. t = 28, d = 8 ; p × 10^2 = 28·57

TABLE IV (b).

Frog's Blood.

(1 part of erythrocytes suspended in ·85% NaCl solution + 2 parts of distilled water.)

1. t = 90, d = 10 ; p × 10^2 = 11·11
2. t = 70, d = 8 ; p × 10^2 = 11·42

TABLE V.

Cat's Blood.

(1 part of erythrocytes suspended in 0·85% NaCl solution + 2 parts of distilled water.)

1. t=122 ; d=106 ; p × 10^2 =86·88
2. t= 62 ; d= 55 ; p × 10^2 =88·71

TABLE VI.

Value of $p \times 10^2$ in the blood of a number of healthy students, the blood being treated with two parts of $\frac{N}{50}$ NaCl (deduced from the author's paper in the Bio-Chemical Journal, Vol. IV, Nos. 5, 6 and 7).

1	2	3	4	5	6
58·40	58·40	60·90	64·30	56·40	56·30

From the above observations the following laws can be deduced :—

1. The amount of hæmoglobin dissolved in a given volume of hypotonic sodium chloride solution or distilled water is proportional to the amount of hæmoglobin in the erythrocytes presented for solution in the form of a suspension.

2. The ratio between these two factors varies in different animals, being the lowest in our table in the case of the frog and highest in the case of the sheep. In the case of the rabbit it is also very high.

3. It is fairly constant in healthy individuals but varies in disease (*vide* chap. III).

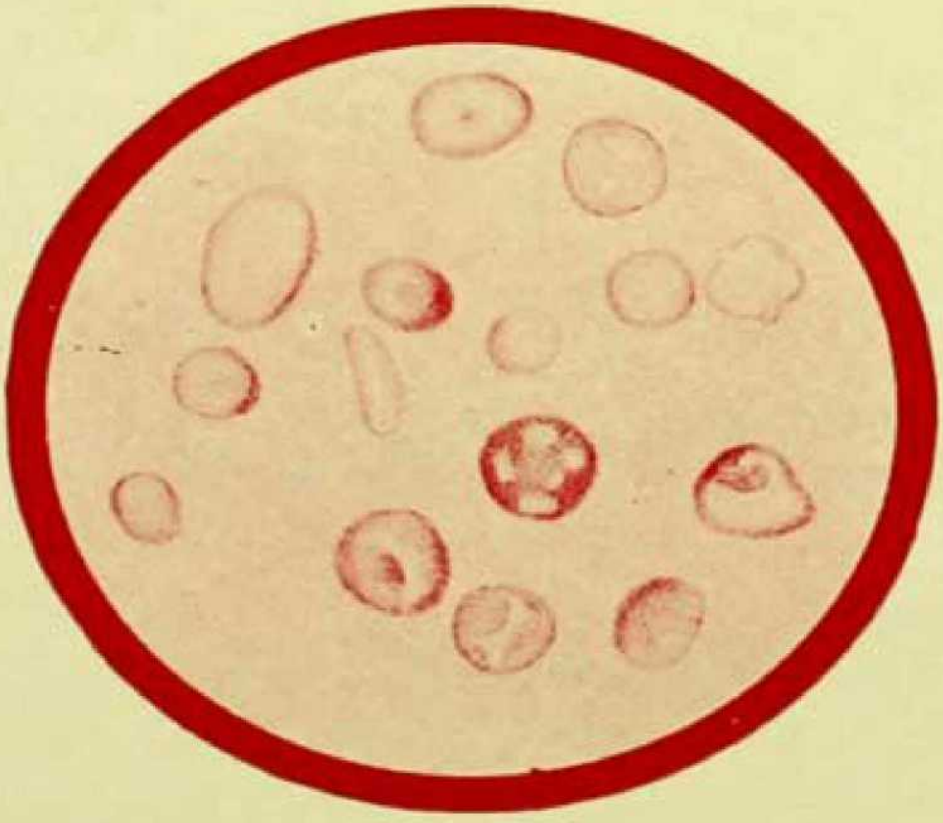
From the above, it will be seen that the same law that regulates the solution of globulin in solution of neutral salts also regulates the solution of hæmoglobin contained within erythrocytes. In other words, the latter follows the law enunciated by Mellanby and Hardy.[1] This fact probably throws some light as to how hæmoglobin exists inside erythrocytes. Each individual corpuscle allows a certain quantity of water to

[1] Journal of Physiology Vol. XXXIII, 1905; also referred to the Proceedings of the Royal Society Series B, Vol. LXXIX, 1907.

enter into its substance by the process of osmosis, when blood is treated with a hypotonic sodium chloride solution or distilled water. If, now, we consider that the hæmoglobin exists in the form of a suspension inside erythrocytes, it follows, from the law of Mellanby and Hardy, that the amount of hæmoglobin that will be dissolved by water entering into the substance of the erythrocytes is proportional to the amount of hæmoglobin in each individual corpuscle. Accordingly the amount of dissolved hæmoglobin that will pass out of the corpuscles is proportional to the amount of hæmoglobin inside them. It follows, therefore, that the amount of hæmoglobin in a suspension of erythrocytes that will dissolve when the suspension is treated with distilled water or hypotonic saline, is proportional to the total amount of hæmoglobin present in them. We can, therefore, conclude that *hæmoglobin as it exists inside erythrocytes is in a state of suspension.*

CENTRAL LIBRARY

PLATE V.



The erythrocytes in a case of anæmia.

(Page 47.)

CHAPTER XI.

Constitution of the erythrocytes revealed from the phenomenon of hæmolysis. Chemical combination and mass action. Mechanism of crenation. Entrance of water into the erythrocytes and their resistance to hæmolysis partly a vital phenomenon.

Under what conditions does hæmoglobin exist in red corpuscles?

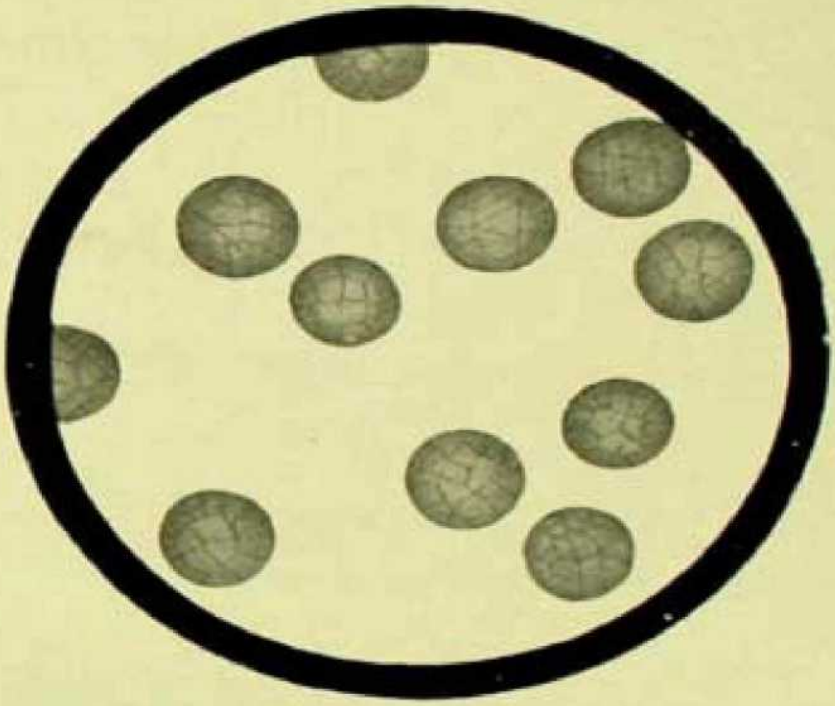
We have already pointed out in the last chapter that hæmoglobin, as it exists inside red corpuscles, is in the form of a suspension.

Further investigations carried on by me lead to the conclusion that there exists a union allied to chemical combination between the hæmoglobin of the erythrocytes and other portions of their structure. If we examine the resistant corpuscles under the microscope it is easily seen that a large number of them have undergone marked changes in shape, size, and in the amount of contained hæmoglobin. The appearances very much resemble what we observe in cases of anæmia (vide plates II and V). Some of the corpuscles are resistant in the sense that they have not at all discharged their hæmoglobin. But there are others which shew marked diminution in the amount of contained hæmoglobin. Some shew changes in the distribution of contained hæmoglobin as compared with the normal. Evidently there has been a partial escape of hæmoglobin from these corpuscles. The question may be asked as to what it is that

prevents the remaining portion of the hæmoglobin from being completely discharged. The most probable assumption is that the process of hæmolysis by hypotonic saline or distilled water is to some extent allied to *mass action* that takes place in chemical reactions. In other words, there probably exists a union allied to chemical combination between the hæmoglobin of erythrocytes and other portions of their structure. *Hæmoglobin, therefore, exists inside erythrocytes in the form of a suspension in combination with some portions of the substance of erythrocytes.*

Another explanation may be offered of the above phenomenon. Let us now consider the structure of the partially hæmolysed erythrocytes. It can be shewn that on addition of a $\frac{N}{10}$ NaCl solution to a sample of blood hæmolysed by distilled water, a turbidity again appears which must be due to the shrinking of the corpuscles. It thus appears that the osmotic capabilities of the cellular framework of these cells have not been altogether destroyed. To consider, therefore, that the erythrocytes are simple bags of membrane containing hæmoglobin rupturing when treated with distilled water seems to be untenable. Such simple bags of membrane when once ruptured will allow water to enter into their structure, when washed with $\frac{N}{10}$ NaCl, till the whole of the hæmoglobin contained inside them is completely washed out. Therefore, the fact that such partially hæmolysed corpuscles contain, when washed with $\frac{N}{10}$ NaCl, some amount of hæmoglobin inside their structure renders the simple bag-theory untenable. On the other hand, we may consider that they consist of specially constructed bags with membraneous partitions containing hæmoglobin, as represented in figure IX. Some of the membranes rupture in the partially hæmolysed corpuscles, while others remain unruptured.

FIG. IX.



Diagrammatic representation of partitioned walls inside red corpuscles.

(Page 48.)

We have already observed that if a suspension of erythrocytes in $\frac{N}{10}$ NaCl solution, is placed over a slide and then allowed to dry gently, we find that the dried corpuscles when treated with $\frac{N}{10}$ NaCl solution, dissolve completely. Let us consider what happens when the erythrocytes are drying on the slide. They tend to stick to one another and to the slide. On the other hand, as evaporation goes on, they tend to contract. As a result of these two antagonistic processes there is perhaps rupture of their membraneous structure, and they dissolve when they come in contact with what would be an isotonic solution in the case of undried erythrocytes. Their complete solution indicates complete disruption of their cellular framework in the process of drying up.

This complete disruption is either purely mechanical due to rupture of all the membraneous portions inside the erythrocytes or the removal of H_2O during evaporation brings about a complete change in the chemical constitution of the erythrocytes converting them into particles soluble in saline of any strength, just as a lump of sugar dissolves in water. This latter view best explains the complete solution of erythrocytes when treated with saturated solution of NaCl in water. In the process of desiccation of the erythrocytes by the saturated NaCl solution water is removed from them. A portion of this water is perhaps in chemical combination with the erythrocytes and this compound is broken up when they are allowed to dry on a slide or are treated with saturated NaCl solution.

Another explanation may be offered to explain why erythrocytes that are drying on a slide dissolve in $\frac{N}{10}$ NaCl solution. When a suspension of the erythrocytes in $\frac{N}{10}$ NaCl solution is

dried over a slide, the NaCl solution tends to become more and more concentrated and consequently a stage comes at which it becomes saturated and hence the erythrocytes come to be in contact with a saturated NaCl solution and then they behave like what have been already described as salted erythrocytes and therefore dissolve when further treated with $\frac{N}{10}$ NaCl solution.

We thus see

1. (Blood + saturated NaCl solution) + $\frac{N}{10}$ NaCl solution = laking.
2. Blood allowed to dry on a slide + $\frac{N}{10}$ NaCl solution = laking.

From these facts we may conclude that hæmoglobin as it exists inside erythrocytes probably exists as a compound with H_2O and that this compound is decomposed by abstraction of water during evaporation or by treatment with very concentrated NaCl solution ($\frac{S}{1}$ or $\frac{S}{2}$).

The above explanation is open to the objection that when the erythrocytes are treated with moderately strong NaCl solutions we find crenation of the corpuscles taking place without hæmolysis.

It is, however, possible that the portion of the H_2O, which is not in chemical combination with hæmoglobin, comes out of the erythrocytes when the latter are treated with moderately strong NaCl solution giving rise to crenation of the corpuscles, while the portion, that is in chemical combination, comes out when the erythrocytes are treated with saturated NaCl solution giving rise to their complete hæmolysis. This compound may be represented as H—X—OH.

The holding of hæmoglobin within the erythrocytes is, to some extent, a *vital phenomenom*. This is partly proved by the phenomenon of spontaneous hæmolysis and partly also by the fact that when one volume of freshly drawn blood mixed with 2 vols. of $\frac{N}{10}$ NaCl solution shews only a faint hæmolysis, the same shews a distinct hæmolysis when treated in the same way after it has been kept shed for 60 to 80 hours. This inadequacy of the erythrocytes, in blood that has been kept shed for some time, to hold hæmoglobin within their substance is independent of any bacterial infection and is probably a sign of commencing loss of vitatily. This loss of vitality takes place much more quickly when the erythrocytes dry up than when they are kept in a closed tube, evaporation being thereby prevented.

We thus conclude that *hæmoglobin exists inside erythrocytes in suspension and in combination with* H_2O. Its existence inside erythrocytes is partly a *vital phenomenon*. In this connection we must also refer to the observations of Moore, who has shewn that the ions inside red corpuscles are attached to the hæmoglobin, the phosphates being firmly held than the chlorides.[1] Similarly, the experiments of Stewart lead to the conclusion that hæmoglobin as it exists inside red corpuscles, is united with the stroma, while a portion of the electrolytes remain in solution as such, another portion being combined with the stroma.[2]

The hæmolysis of erythrocytes by hyposmotic or hyperosmotic NaCl solutions :—

We just now stated that hæmoglobin exists inside corpuscles in combination with H_2O and also the phosphates and the chlo-

[1] Further Advances in Physiology.

[2] Journal of Physiology, Vol. XXIV.

rides. If we assume that this compound of hæmoglobin is decomposed by excess of water or by a very hypertonic NaCl solution or during evaporation, then the phenomenon of hæmolysis can be explained without assuming that the walls of the erythrocytes are ruptured during hæmolysis. When water enters the erythrocytes in excess, this compound of hæmoglobin is decomposed and dissolves in the excess of water and this dissolved hæmoglobin comes out of the erythrocytes by a process allied to filtration or as Stewart suggests, even by diffusion,[1] though hæmoglobin is non-dialysable through ordinary membranes. If the water entering the erythrocytes is not in excess, then there is only distension of the erythrocytes but not decomposition of the compound of hæmoglobin. In other words, this compound is decomposed only in excess of water. Similarly when the erythrocytes are treated with saturated or half saturated NaCl solution, then also the same compound is decomposed and the erythrocytes behave like spheres of sponges containing dissolved colouring matter and hæmoglobin comes out of them as through a filter. *In other words, this compound is decomposed by excess of water as well as by excessive abstraction of water.*

The amount of hæmoglobin that will come out of an individual erythrocyte during the process of laking by hyposmotic NaCl solution will depend upon the hypothetical compound of hæmoglobin that is decomposed and this depends upon the masses of the interacting compounds, namely, the compound of hæmoglobin and the water that enters the erythrocytes. This latter depends greatly upon *osmosis* and to some extent upon the permeability of the outer portion of the erythrocytes to water.

[1] Journal of Physiology, Vol. XXIV.

This permeability, as has been already pointed out, is not always the same. The behaviour of crenated corpuscles towards $\frac{N}{10}$ NaCl solution and the presence of crenated corpuscles in the blood of some forms of anæmia, prove that the permeability of erythrocytes to water is variable. Crenation probably means commencing loss of vitality of the corpuscles and is associated with diminution of their permeability to water. This diminution evidently takes place in spite of the force of *osmosis*. Crenation, therefore, is not simply due to ex-osmosis of water from the corpuscles, as is generally supposed to be the case. If the latter were the only cause of crenation, then one would expect to observe the greatest amount of crenation when blood is treated with saturated NaCl solution, but I have shewn that this is not the case.

We have, however, already pointed out that, under normal circumstances, this permeability is the same in all the corpuscles. But in abnormal conditions *e.g.*, during crenation and also perhaps in disease this permeability of the corpuscles is not the same as that of the normal ones.

It is a law in physical chemistry that osmosis is independent of the membranes through which it takes place. As the permeability of corpuscles varies under different conditions, it is doubtful whether the law holds good absolutely in their case. It is, on the other hand, likely that they possess a *specific permeability* which is the same in normal conditions but may vary under abnormal conditions. This *specific permeability* should be a subject for further investigations. Apart from osmosis, the amount of water that would enter the erythrocytes, is determined by the activity of the outer portion of the erythrocytes, which

constitutes their specific permeability and which is probably dependent upon their *vitality*. Similarly, resistance to hæmolysis is, to some extent, dependent upon the same cause. Permeability of erythrocytes to water, is, to some extent, comparable to the absorption of oxygen into the lung tissue which is partly due to epithelial activity, as has been shewn by the experiments of Bohr and Haldane. The existence of this specific permeability is also proved by the experiments of Moore and Roaf[1] shewing that the freezing points of the corpuscles and the serum are not exactly the same and this could not be the case if there were a perfect osmotic equilibrium between the corpuscles and the serum as one would expect to find if the walls of the erythrocytes behaved like ordinary membranes.

[1] Bio-Chemical Journal, 1908.